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Description of a new *Peronia* species (Gastropoda: Eupulmonata: Onchidiidae) from Iran, Persian Gulf

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Abstract

Peronia J. Fleming, 1822 is an eupulmonate slug genus with a wide distribution in the Indo-Pacific Ocean. Currently, nine species are considered as valid. However, molecular data indicate cryptic speciation and more species involved. Here, we present results on a new species found in the Persian Gulf, a subtropical region with harsh conditions such as elevated salinity and high temperature compared to the Indian Ocean. *Peronia persiae* **sp. nov.** is described based on molecular, histological, anatomical, micro-computer tomography and scanning electron microscopy data. ABGD, GMYC and bPTP analyses based on 16S rDNA and cytochrome oxidase I (COI) sequences of *Peronia* confirm the delimitation of the new species. Moreover, our 14 specimens were carefully compared with available information of other described *Peronia* species. *Peronia persiae* **sp. nov.** is distinct in a combination of characters, including differences in the genital (ampulla, prostate, penial hooks, penial needle) and digestive systems (lack of pharyngeal wall teeth, tooth shape in radula, intestine of type II).

Key words: Histology, species delimitation, phylogeny, *Peronia verruculata*, Systellommatophora, Mollusca

Introduction

The usually shallow Persian Gulf (average depth 50 m), located in the subtropical northwest of the Indian Ocean, is exposed to harsh conditions and limited exchange of water through the Strait of Hormuz. Sea surface temperature ranges from 24 to 32°C in the Strait of Hormuz and 16 to 32°C in the extreme northwest, with a short cold spring and a long warm summer season (Amini Yekta *et al*. 2013). Seawater salinity ranges from 37 to 38 ppt in the entrance of the Gulf up to 38 to 41 ppt in the extreme northwest, and the range of the tides varies according to the locality from 1.4 m up to 3.2 m (Evans 1999). This contrasts the typical water conditions of the Indian Ocean, with sea surface temperature ranges of 22.2 to 27.7°C (Sawe 2018), and a lower salinity range of 36.5−37.2 ppt (Pous *et al*. 2015). Therefore, the Persian Gulf differs from the Indian Ocean regarding these physiochemical parameters and an impact on the diversity and ecology especially of the intertidal fauna is very likely. However, almost no biodiversity studies exist from this region. Only a few ones documented the occurrence of marine Heterobranchia along the south Iranian shorelines (Amini Yekta *et al*. 2012; Amini Yekta *et al*. 2013; Attaran *et al.* 2015, Rezai *et al.* 2016), with a single species of the eupulmonate Onchidiidae recorded: *Peronia peronii* (Cuvier, 1804), identified either by external morphology (Amini Yekta *et al*. 2012) or by 18S rDNA sequences in comparison to other heterobranchs (Attaran *et al.* 2015).

Members of the Onchidiidae, a family within the eupulmonate group Systellommatophora, have a widespread distribution and are quite common in the middle and upper intertidal zone, either in rocky, muddy or sandy habitats (Dayrat 2009; Zhang *et al*. 2016). Most species are exclusively found in tropical and subtropical areas of the Indo-West Pacific (Wu *et al*. 2010). Onchidiids have no shell and the notum can be smooth or covered with tubercles in different sizes and shapes. Several characters are typical for certain genera, others rather unique. Multiple photoreceptors including dorsal eyes on the notum, for example, are characteristic for the genera *Onchidium* Buchannan, 1800, *Peronina* Plate, 1893, *Melayonchis* Dayrat & Goulding, 2017, *Wallaconchis* Goulding & Dayrat, 2018,

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Alionchis Goulding & Dayrat, 2018, and *Marmaronchis* Dayrat & Goulding, 2018, but are absent in *Onchidella* J.E. Gray, 1850, and *Onchidina* Semper, 1882. Branchial gills on the notum are typical for *Peronia*. In former times, they were also reported for *Paraperonia* Starobogatov, 1976 (now accepted as synonym of *Peronia*, Dayrat 2009) and *Scaphis* Starobogatov, 1976.

Onchidiidae have been recognized since 1815 as a natural taxon and its monophyly was confirmed in recent studies (Dayrat 2009; Dayrat *et al*. 2011). However, the relationships of genera and between species remain poorly understood. Furthermore, species identification is hampered by the lack of distinct external features. Although the genus *Peronia* has nine currently accepted species (MolluscaBase, 2018) and is one of the oldest described onchidiid genera, only a few additional studies besides species original descriptions are available. *Peronia verruculata* (Cuvier, 1830) was thoroughly investigated by Awati & Karandikar (1948) using histological methods, but unfortunately, they did not mention the locality of the specimens they investigated. The type locality of *P. verruculata* is mentioned with India, Bandra, and the species seems to have a very wide distribution in the Indo-Pacific Ocean (GBIF, 2019). Further information for this species was provided by Plate (1893), Labbé (1934), Britton (1984) and Hyman (1999). *P. peronii* was re-investigated only a few times (Labbé 1934; Plate 1893) and additional information on the other seven described species is rarely available. The synonymization of various genera with *Peronia* has rather added to the confusion surrounding this clade, for example *Quoyella* Starobogatov, 1976 and its monotypic species *Q. indica* Labbé, 1934 (now *Peronia indica*). In this case, the possession of branchial gills led to the synonymization with *Peronia*, while other morphological characters i.e. two separate male openings and intestinal type five would not support the species in the genus *Peronia* (Table S1).

In this study, we address the *P. verruculata* complex by re-analysing all available literature data and analysing all available sequences from NCBI together with new sequences from Iranian specimens. We describe a new species from the Persian Gulf based on an integrative morphological and molecular approach and show its distinctiveness in morphology from all other valid *Peronia* species, including the close related species *P. verruculata*.

Material and methods

Sampling was undertaken in the intertidal zone during low tide at Bandar Lengeh (26°33′29″N 54°52′50″E) in March 2015, and Lavan Island (26°48′20.99″N 53°16′4.80″E) in February 2016 (Figure 1). In total, 14 specimens of *Peronia* sp. were collected in the intertidal zone from the surface of rocks. Some of them were hidden in rock crevices but could be traced by the trails produced during feeding. The animals were photographed alive using a digital camera Canon SX160IS and measured for length. For details of locality and preservation method see Table 1. Temperature, pH value and salinity were measured at Lavan Island in 2016.

FIGURE 1. Collecting sites of *Peronia persiae* **sp. nov. A.** Persian Gulf, general view. **B.** Rocky shore where the specimens were collected during low tides.

TABLE 1. Specimens used in this study. Abbreviations: BL=Bandar Lenge; LA=Lavan Island.

Specimen	**Preservation**	**Purpose**	**Length of preserved animal (mm)**
BL 1	ETOH 96%	**Paratype;** DNA barcoding, SEM, dissection	37
BL 2	Formalin	Histology	32
LA 1	ETOH 96%	**Paratype;** DNA barcoding, SEM, dissection	32
LA 2	ETOH 96%	**Paratype;** DNA barcoding, SEM, dissection	22
LA 3	ETOH 96%	Voucher; DNA barcoding, SEM, dissection	26
LA 4	ETOH 96%	Voucher; DNA barcoding, SEM, dissection	28
LA 5	ETOH 96%	Voucher; DNA barcoding, SEM, dissection	34
LA 6	ETOH 96%	Voucher; DNA barcoding, SEM, dissection	32
LA 7	ETOH 96%	**Holotype**; DNA barcoding, SEM, dissection of head area	35
LA 8	ETOH 96%	SEM, DNA barcoding, dissection	25
LA 9	ETOH 96%	SEM, DNA barcoding, dissection	22
LA 10	ETOH 96%	SEM, DNA barcoding, dissection, Histology of genital system	13
LA 11	Formalin	Histology	29
LA 12	ETOH 96%	Micro CT, dissection	31

Anatomical observation. Dissection was performed under an Olympus SZX12 stereo microscope. Radula, penis, and needle of penial accessory gland were extracted by using a 5% KOH solution, dried and analysed using a Scanning Electron Microscope (ZEISS Sigma VP 300). For Micro-CT analyses one specimen was stained for two days in 1% iodine dissolved in 100% Ethanol (I2E). Reconstructed images were analysed in CTVox. For histological analyses, two specimens and the genital system of an additional specimen were dehydrated in EtOH and embedded in hydroxyethyl methacrylate (Heraeus Kulzer GmbH) for serial sectioning. Sections (2.5 µm) were stained with toluidine blue, photographed subsequently under a ZEISS Microscope (Imager.Z2m) and analysed with ZEN software (ZEISS) at Alexander Koenig Research Museum in Bonn, Germany.

DNA extraction, PCR, and DNA sequencing. DNA isolation was carried out using the Qiagen DNeasy Blood and Tissue kit, following manufacturer's instructions. Partial sequences of mitochondrial COI (ca. 680 bp) and ribosomal 16S (ca. 550 bp) were amplified by polymerase chain reaction (PCR) using the primers LCOI490-JJ (5'-CHACWAAYCATAAAGATATYGG-3') and HCO2198-JJ (5'-AWACTTCVGGRTGVCCAAARAATCA-3') (Astrin & Stüben 2008) for COI; 16Sar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5'- CCGGTCTGAACTCAGATCACGT-3') (Palumbi *et al*. 1991). The thermoprofile during the PCR used for COI and 16S was: 15 min at 95°C; 40 cycles of reaction conditions (48 cycles for 16S) involved an initial denaturation of 94°C for 35sec (45 sec for 16S), subsequent annealing at 55°C for 90 sec (56°C for 45 sec for 16S), elongation at 72°C for 90 sec and final elongation step of 72°C for 10 min. Sequencing was performed by Macrogen Europe (Amsterdam, Netherlands). Sequences are deposited in GenBank with the accession numbers listed in Table 2.

TABLE 2. Name of species, locality information, COI and 16S GenBank accession numbers for genus *Peronia*. # Registered but unpublished data of specimens taken from NCBI.

Preliminary name taken from literature (NCBI)	**New assigned / Confirmed name**	**Locality**	**GenBank COI**	**GenBank 16S**
Scaphis sp.		Philippine Islands	HQ660050	HQ659918
Onchidium verrucosum	*Peronia verruculata* (misspelling by Göbbeler and Klussmann-Kolb 2011)	Australia (Queensland)	EF489391	EF489316
Peronia sp. 1		Hawaii	HQ660038	HQ659906

.....continued on the next page

TABLE 2. (Continued)

Preliminary name taken from literature (NCBI)	**New assigned / Confirmed name**	**Locality**	**GenBank COI**	**GenBank 16S**
Peronia sp. 2		Oman	HQ660044	HQ659912
Peronia sp. 3		Queensland (Australia)	HQ660048	HQ659916
Peronia sp. 4		Mozambique	HQ660045	HQ659913
Peronia sp. 5		Mozambique	HQ660047	HQ659915
Peronia sp. 6		Indonesia (Sulawesi)	HQ660046	HQ659914
Peronia sp. 7		Hainan (China)	HQ285979	HQ285967
Peronia sp. 8		Hainan (China)	HQ285980	HQ285968
Peronia sp. 9		Hainan (China)	HQ285981	HQ285966
Peronia sp. (unpublished paper by Chen *et al.* 2011)	*Peronia verruculata* (reassigned by Sun *et al.* 2014)	Hainan (China) #	JN543165	JN543101
Peronia cf. *verruculata* (Dayrat *et al.* 2011)	*Peronia* sp. (reassigned by Dayrat *et al.*2016)	Okinawa (Japan)	HQ660043	HQ659911
Peronia peronii		Guam	HQ660041	HQ659909
Peronia cf. *peronii*		Mozambique	HQ660042	HQ659910
Peronia verruculata (unpublished paper by Wang *et al*. 2009)		Fujian (China) #	GU166568	-
		Fujian (China) #	GU166567	-
		Fujian (China) #	GU166566	GQ985284
		Fujian(China) #	GU166564	GQ985282
		Fujian(China) #	GU166563	GQ985281
		Hainan(China)#	GU166561	GQ985277
		Hainan(China) #	GU166559	GQ985275
		Hainan(China) #	GU166558	GQ985274
		Hainan(China) #	GU166562	GQ985278
		Hainan(China) #	GU166560	GQ985276
		Fujian(China) #	GU166565	GQ985283
		Hainan(China) #	GU166557	GQ985273
Peronia verruculata (unpublished paper by Chen *et al*. 2011)		Fujian (China) #	JN543154	JN543090
		Hainan (China) #	JN543153	JN543089
		Zhanjiang (China) #	JN543152	JN543088
Peronia sp.		Singapore	MH002607	-
			MH002590	-
			MH002605	-
			MH002603	-
			MH002600	-
			MH002599	-
			MH002594	-
			MH002591	-
			MH002589	-
			MH002586	-
			MH002585	-
			MH002580-MH002582	-
			MH002575-MH002578	-

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TABLE 2. (Continued)

Preliminary name taken from literature (NCBI)	**New assigned / Confirmed name**	**Locality**	**GenBank COI**	**GenBank 16S**
			MH002592	-
			MH002596	-
			MK142731	-
			MK142730	-
			MK142725-MK142728	-
			MK142720-MK142723	-
			MK142717	-
			MK142715	-
			MK142714	-
			MK142712	-
			MK142704	-
			MK142706-MK142709	-
			MH002597	-
			MH002604	-
			MH002579	-
			MH002598	-
			MH002602	-
			MH002583	-
			MK142710	-
			MH002593	-
			MK142729	-
			MK142713	-
			MH002595	-
			MK142732	-
			MK142724	-
			MK142716	-
			MH002588	-
			MK142705	-
			MK142719	-
			MK142711	-
			MH002584	-
			MH002606	-
			MH002601	-
			MK142718	-
Peronia sp. 2		Singapore	MK142680-MK142703	-
			MH002574	-
			MH002573	-
			MH002569	-
			MH002567	-
			MH002566	-
			MH002559-MH002563	-

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TABLE 2. (Continued)

Preliminary name taken from literature (NCBI)	**New assigned / Confirmed name**	**Locality**	**GenBank COI**	**GenBank 16S**
			MH002548-MH002556	-
			MH002546	-
			MH002545	-
			MH002571	-
			MH002572	-
			MH002547	-
			MK142694	-
			MK142689	-
			MH002564	-
			MH002550	-
			MH002557-MH002558	-
			MH002570	-
Peronia persiae **sp. nov.**		Bandar Lenge	MK312166	MK312167
		Lavan Island	MK993386-MK993395	MK993398-MK993407
Peronia sp. 7		Indonesia, Bangka Island	MK993396	MK993397

Phylogenetic reconstruction. Sequences were edited using BioEdit (ver.7.2.6.1) (Hall 1999) and aligned using MAFFT (Katoh *et al*. 2002) in Geneious v7.1.9 (Kearse *et al*. 2012). Sequences of other species from all available genera of the family Onchidiidae (446 sequences) and outgroup (three sequences) were downloaded from GenBank, from Wang *et al*. (2009), Chen *et al.* (2011), Dayrat *et al*. (2011, 2014, 2016, 2017, 2018, 2019), Dayrat & Goulding (2017); Cumming *et al.* (2014), Goulding *et al.* (2018a, 2018b, 2018c) and Layton *et al*. (2014) (Table 2). All sequences were included in the phylogenetic analysis to evaluate the affiliation and monophyly of *Peronia* for subsequent analyses. Two species of *Siphonaria* G. B. Sowerby I, 1823 (*S. zelandica* Quoy & Gaimard, 1833 and *S. sirius* Pilsbry, 1894), considered as the closest relatives to the Onchidiidae (Wägele *et al*. 2014) were included as outgroup for rooting the tree. After trimming, the alignments comprised 454 bp for 16S, 635 bp for COI and 1075 bp for the concatenated dataset. The maximum likelihood (ML) analysis was run in IQ-TREE (Nguyen *et al*. 2014; Trifinopoulos *et al*. 2016) based on the concatenated data set using the online version 1.6.3 on a webserver (http://iqtree.cibiv.univie.ac.at/), with the GTR model for each gene. Support values were calculated based on 1000 ultrafast bootstrap replicates and the approximate likelihood ratio test (SH-aLRT) (2000 replicates). Subsequent analyses were performed with a reduced and concatenated alignment, only including *Peronia* sequences (Table 2), with identical sequences removed. For this analysis, *Wallaconchis graniferus* (Semper, 1880) was chosen as outgroup, based on the results of the general Onchidiidae analysis. Species delimitation tests were performed with the same taxa, but with 16S and COI genes datasets separated, and the results presented on the COI (Figure 11) and the concatenated trees (Figure 12). Dendroscope (version 3.5.8) (Huson & Scornavacca 2017) and Inkscape (version 0.92) (https://inkscape.org/en/) were used to edit the phylograms.

Species delimitation. The Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.* 2011), General Mixed Yule Coalescent model (GMYC) (Monaghan *et al.* 2009; Pons *et al.* 2006) and Poisson Tree Processes (PTP) (Zhang *et al.* 2013) were applied for delimiting the species. ABGD is independent of predefined species groups (Puillandre *et al.* 2011; Padula *et al.* 2014) and was applied to COI and 16S datasets separately and as concatenated datasets, using default values under the Kimura, K80model. GMYC is a likelihood method for delimiting species by fitting within- and between-species branching models on phylogenetic trees (Pons *et al*. 2006; Monaghan *et al.* 2009). bPTP is an updated version of the original maximum likelihood PTP program (maximum likelihood PTP search result is part of the bPTP results) and adds Bayesian support (BS) values to delimited species on the input tree (Figures S1–S2) (Zhang *et al.* 2013). Ultrametric starting trees for GMYC were generated using penalized likelihood (Sanderson 2002) using the chronos function in the APE package (Paradis *et al.* 2004) in R. Four clock

models: strict, discrete rate variation with ten rate categories, correlated and uncorrelated-relaxed were fitted on the ML trees for COI and 16S using the chronos function in the APE package (Paradis *et al.* 2004). The best model was selected using the phi information criterion, which takes the penalized term into account (Paradis 2013). All models were fitted for trees based on both COI and 16S with the smoothing parameter, lambda, set to 0.1, 0.5 and 1.0, and in each case, the strict clock was found to be the best model. Ultrametric trees for both genes based on a strict clock with lambda=1 were used in subsequent analyses. Single threshold GMYC analyses were performed in R using the SPLITS package (Ezard *et al.* 2009). The PTP model assumes that the number of substitutions is significantly higher between species than within species, which is reflected in the branch lengths (Zhang *et al.* 2013). bPTP analyses were run on COI and 16S trees resulting from the ML analyses and uploaded, individually, as Nexus files to the PTP web server (http://species.h-its.org/) (Zhang *et al.* 2013). Trees were rooted and *W. graniferus* was included as outgroup. bPTP graphic results for each gene were presented as PhyloMaps (Zhang *et al.* 2011). COI minimum and maximum pairwise uncorrected p-distances, between and within the main clades, were calculated using Species Identifier (Meier *et al.* 2006).

Results

Systematics

Family Onchidiidae Rafinesque, 1815

Genus *Peronia* J. Fleming, 1822

Type species *Peronia peronii* (Cuvier, 1804)

Diagnosis. Presence of contractible gills distributed irregularly at least in the posterior part of the dorsal notum. Notum always narrower than the pedal sole; warty with dorsal eyes single or in groups. Some species characterized by spicules in the integument. Intestine usually of type I or type II. Rectal gland absent. Penis short with many spines. Penial gland very long with a large muscular sac. Needle apparatus in penial gland sac present (Labbé 1934; Britton 1984; Dayrat 2009).

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(Figs. 2–10)

ZooBank LSID: urn:lsid:zoobank.org:pub:2F2B0734-03E2-4D94-A72D-9E43A132D1DE

Type material. Holotype: Zoologische Staatssammlung München (ZSM Mol 20180017), Lavan Island (26°48′20.99″N 53°16′4.80″E), collected in February 2016, 35 mm in length. Paratypes: Zoologische Staatssammlung München (ZSM Mol 20180018), Bandar Lengeh (26°33′29″N 54°52′50″E), collected in March 2015 (1 specimen), 32 mm in length; Lavan Island (26°48′20.99″N 53°16′4.80″E), collected in February 2016 (2 specimens), 22 and 37 mm in length.

Etymology. *Peronia persiae* **sp. nov.** is named after the home country of the first author, Iran (Persia).

External morphology (Figure 2). Living specimens with 20–65 mm in length, and 13–37 mm when preserved in formalin. Elongate while moving, but more oval to round in outline when resting. Notum of living animals muddy green to grey in colour and covered with tubercles (papilla) (Figure 2A–B). Foot elongate, smooth and light green; covered by hyponotum (Figure 2A). Eight to 16 tubercles bearing dorsal eyes in groups of two to four (Figure 2C). In posterior region of the mantle, six to 16 irregularly branched branchial gills (so called gill-trees) (Figure 2D). Gills expanding only when the animal is submerged. Two retractable tentacles with an apically lying eye between mantle and foot in the head region. Ventrally lying mouth surrounded by oral lips. Male genital pore between right tentacle and labial palp (Figure 2A). Anus located ventrally at the posterior end of the body in the middle between mantle and foot; female opening on the right side of the anus; pneumostome posterior to the anus and opening only when the slug is out of the water.

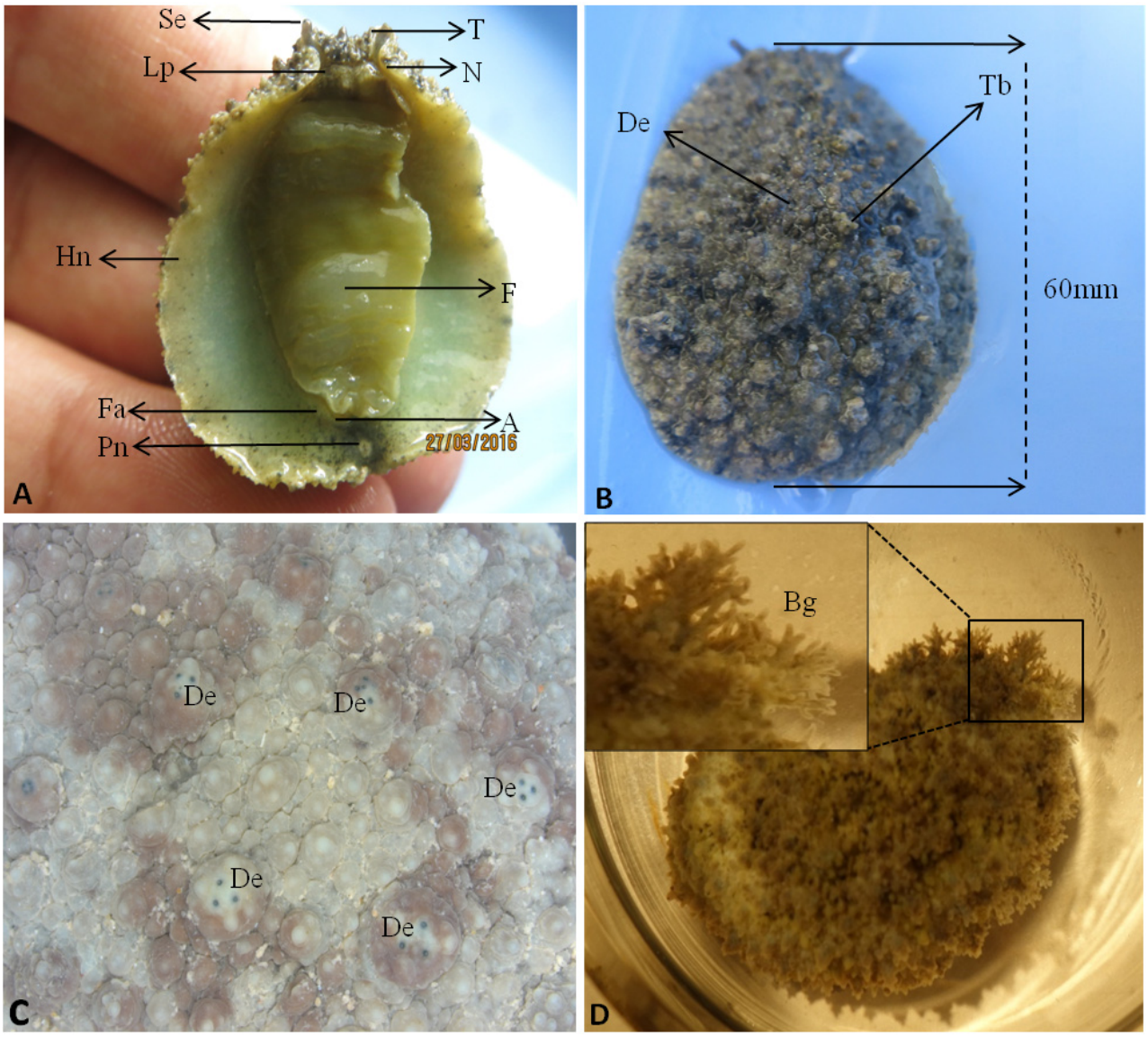

FIGURE 2. *Peronia persiae* **sp. nov.** External characters. **A–B.** Ventral and dorsal view of *Peronia persiae* **sp. nov. C.** Tubercles with dorsal eyes. **D.** Branchial gill at the rear of the back. Abbreviations: A=anus; Bg=branchial gill; De=dorsal eye; F=foot; Fa=female genital aperture; Hn=hyponotum; Lp=labial palp; N=notum; Pn=pulmonary aperture; Se=stalk eye; T=tentacle bearing eye; Tb=dorsal tubercle.

Integument. Epidermis composed of small cells; completely covered by a thin cuticular-like layer. Many bluish stained glandular cells present, reaching deep into the notum tissue; glandular cells staining violet (mucopolysaccharides) interspersed. Some cells without distinct contents (probably empty gland cells), also reaching into notum tissue (Figure 3A).

Vision system. Oval shaped stalk eye on the tip of each tentacle; consisting of cornea, lens and pigmented retina layer (Figure 3B). The latter comprising a villous, pigmented layer, a somatic layer and neural layer with nerve fibres. The dorsal eyes on the notum of inverse nature: the position of the retinal layer inverted with the main nerve penetrating the pigment layer surrounding the retina. Lens composed of two parts: Principal lens consisting of one cell with a large nucleus and thick microvilli layer facing towards epidermis. Accessory lens lying beneath principle lens; formed by at least one cell with a large nucleus. Epidermis covering dorsal eyes similar to surrounding epidermis, but with smaller cells above the eyes (Figure 3C). Photoreceptors as extraocular structures scattered beneath the epidermis, partly single or forming clusters of up to eight receptors and sometimes in close vicinity to dorsal eyes. Each receptor composed of a cell with a thick layer of microvilli and large nucleus. No retina or cornea observed. Photoreceptors separated by connective tissues and probably muscle cells (Figure 3D).

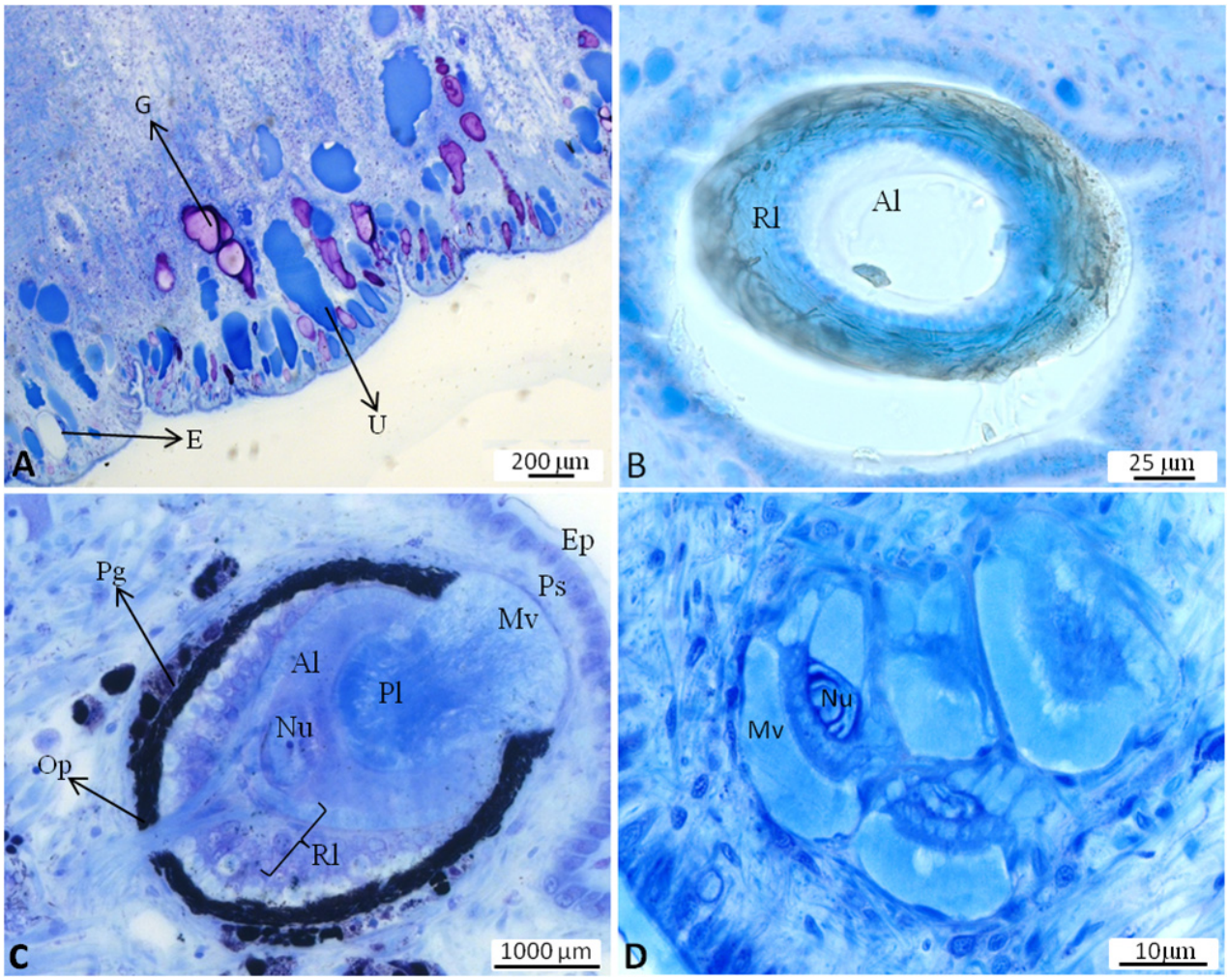

FIGURE 3. Histological sections of epidermis and sensory organs of *Peronia persiae* **sp. nov. A.** Cross section of the mantle. **B.** Cross section of stalk eye. **C.** Longitudinal section of dorsal eye. **D.** Cross section of four dermal photoreceptors. Abbreviations: Al=accessory lens; E=empty unicellular gland; EP=epidermis; G=glandular vesicle consist of mucopolysaccharides (stained violet); U=unicellular gland; Mv=microvillus; Nu=nuclei; Op=optic nerve; Pg=pigmented layer; Pl=principal lens; Ps=pseudo cornea; Rl=retina layer.

Digestive system. Radula broad, with teeth lying on a thick cuticular membrane (Figure 4A). Interior of odontophore filled with red staining substance reminding of hyaline material or connective tissue ("elastic non-cellular substance", Awati & Karandikar 1948, p. 14). Odontophore flanked by the lateral cartilage-like radula cushions as well as pharyngeal muscles; radula cushions connected by a rigid area probably consisting of red stained connective tissue (Figure 4A). Radular formulae counted for 11 specimens; formulae ranging from 49 × 47.1.47 (specimen length alive 22mm) up to 71 × 87.1.87 (specimen length alive 65 mm). Rachidian teeth tricuspid with a main middle cusp and one distinctly narrow and long lateral cusp on both sides of main cusp (Figure 5A). Height of rachidian teeth about 50 µm. First inner lateral teeth smaller than other laterals (Figure 5B); height of unicuspid lateral teeth gradually increasing from about 60 µm to 75 µm and decreasing again towards the lateral rim. Lateral teeth with a conical shape seen from lateral view and inner side curved in a concave way (Figure 5C). Tips (seen from above) usually broadened and with a blunt, spatulate apex (Figure 5A–B). Salivary glands on both sides of the oesophagus with small ducts leading into pharynx close to the transition into the oesophagus; composed of many finger-like tubes combining into clusters and forming a grape-like structure. Glandular cells with reddish to violet staining granular contents (Figure 4B). Oesophagus with highly folded epithelium composed of ciliated columnar cells, covered in many areas with a thin homogenously staining (probably cuticular) layer—sometimes more greenish, sometimes bluish (Figure 4C). Oesophageal folds underlain with red stained connective tissue and surrounded first by a longitudinal and then by a circular muscular layer. Oesophagus entering the first part of the stomach. Stomach

consisting of four parts. First part characterized by a thin epithelium; receiving the ducts of the dorsal and left lateral lobes of the digestive gland. Second chamber strongly muscular (Figure 4D), swollen and stratified; receiving the duct of the posterior lobe of the digestive gland. Third chamber funnel-shaped; pigmented on the outer side; its epithelium forming a highly folded structure with dendritic branches filling nearly completely the internal lumen (Figure 4E). The last chamber representing a small widened section at the beginning of intestine with only thin ridges internally and ciliated cells in the epithelium. Intestine forming two distinct long loops lying close together (Figure 6A–C), thus fitting best type II according to the definition of Labbé (1934). Epithelium with light blue stained cells and with violet stained goblet cells (Figure 4F). Digestive gland composed of many lobes; epithelium exhibiting cells which excrete substances stained in various shades of violet to blue (Figure 4G).

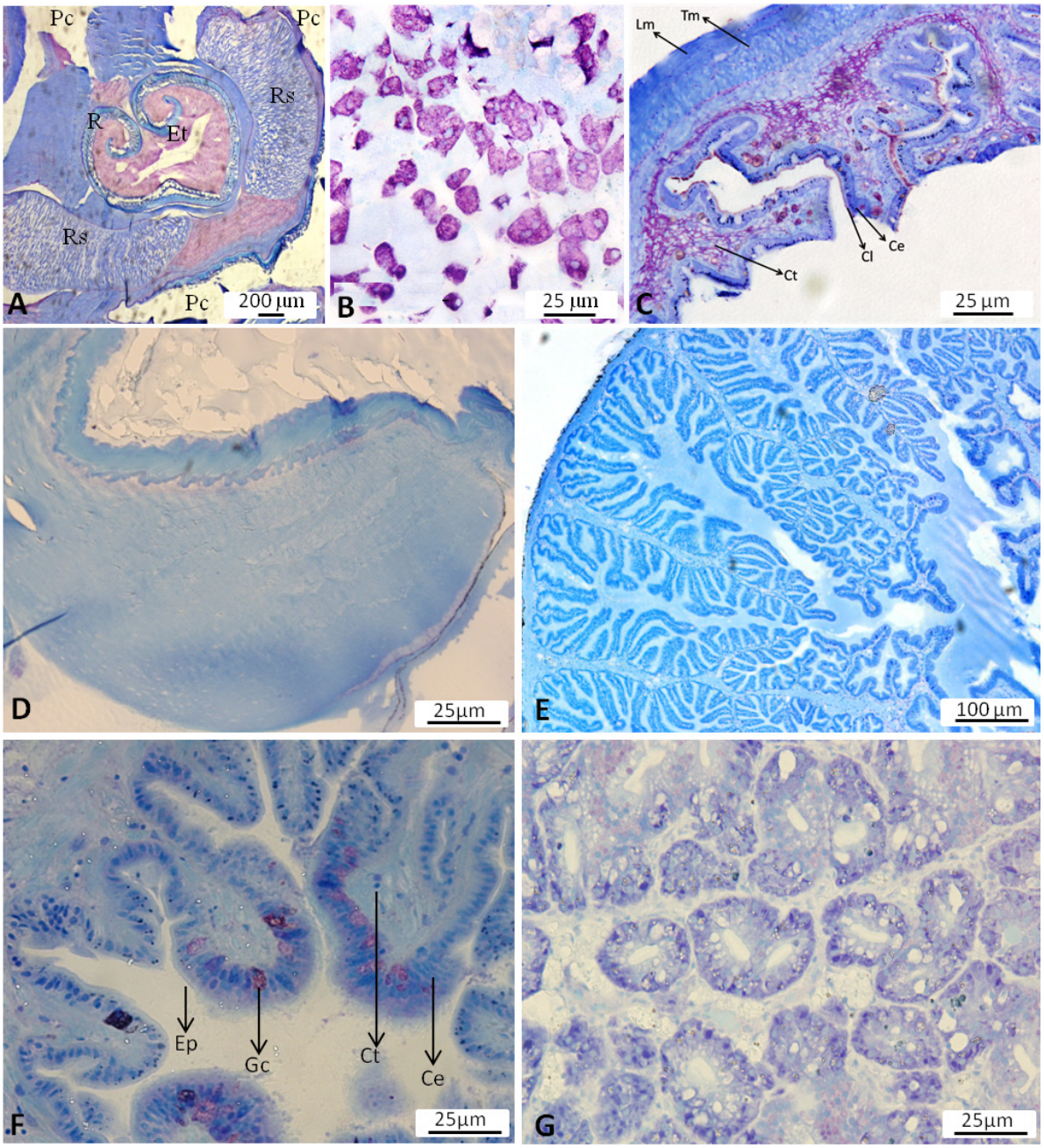

FIGURE 4. Histological sections of the digestive tract of *Peronia persiae* **sp. nov. A.** Cross section through the posterior region of the pharyngeal cavity and radula. **B.** Salivary gland cells. **C.** Cross section of oesophagus. **D.** Second chamber of stomach. **E.** Third chamber of stomach with highly folded (branched) epithelium. **F.** Cross section of intestine. **G.** Cross section of digestive gland. Abbreviations: Ce=columnar epithelium; Cl=cuticular lining; Ct=connective tissue ; Ep=epithelium; Et=non-cellular elastic tissue; Gc=goblet cells; Pc=pharyngeal cavity; R=radula bearing teeth; Rs=radula support; Tm=transverse muscle; Lm=longitudinal muscle.

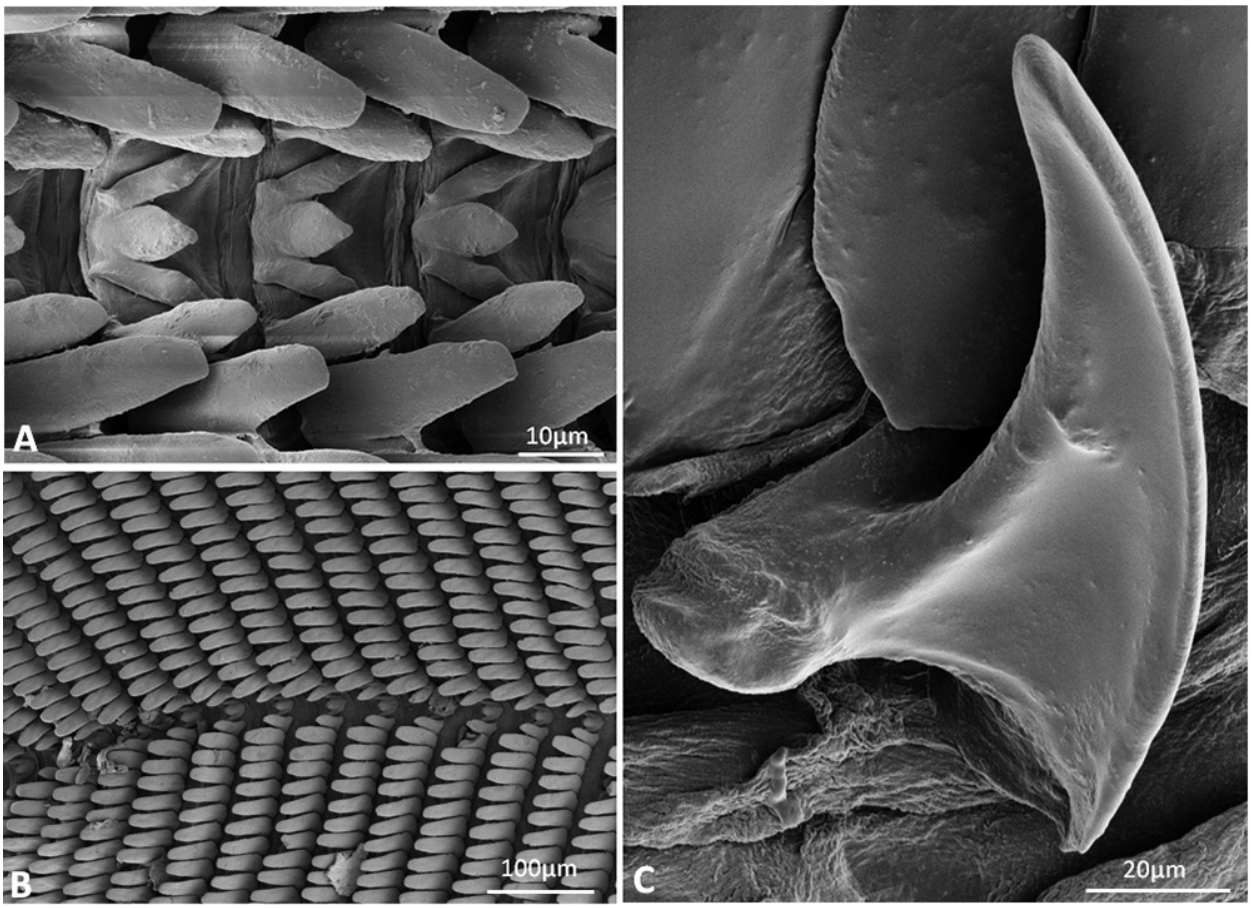

FIGURE 5. Scanning electron micrographs of the radula of *Peronia persiae* **sp. nov.**: **A.** Rhachidian teeth. **B.** Lateral teeth. **C.** Lateral tooth.

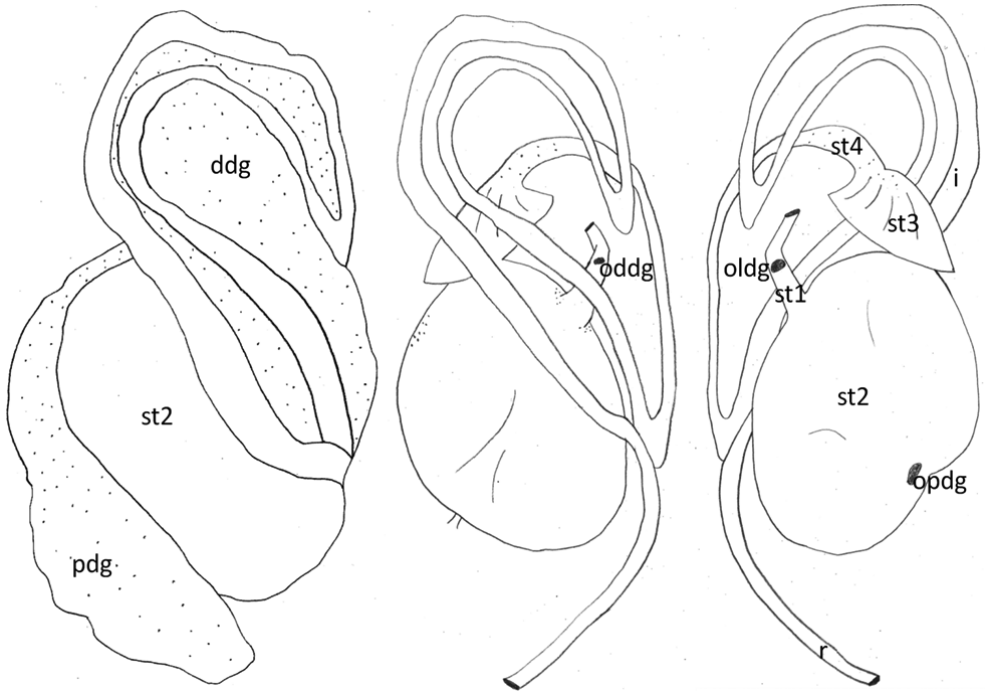

FIGURE 6. Digestive system of *Peronia persiae* **sp. nov. A.** Dorsal view. **B.** Dorsal view with digestive gland removed. **C.** Ventral view. Abbreviations: ddg=dorsal lobe of digestive gland; i=intestine; oddg=opening of dorsal lobe of digestive gland; oldg=opening of lateral lobe of digestive gland; opdg=opening of posterior lobe of digestive gland; pdg=posterior lobe of digestive gland; r=rectum; st1=stomach chamber 1; st2=stomach chamber 2; st3=stomach chamber 3; st4=stomach chamber 4.

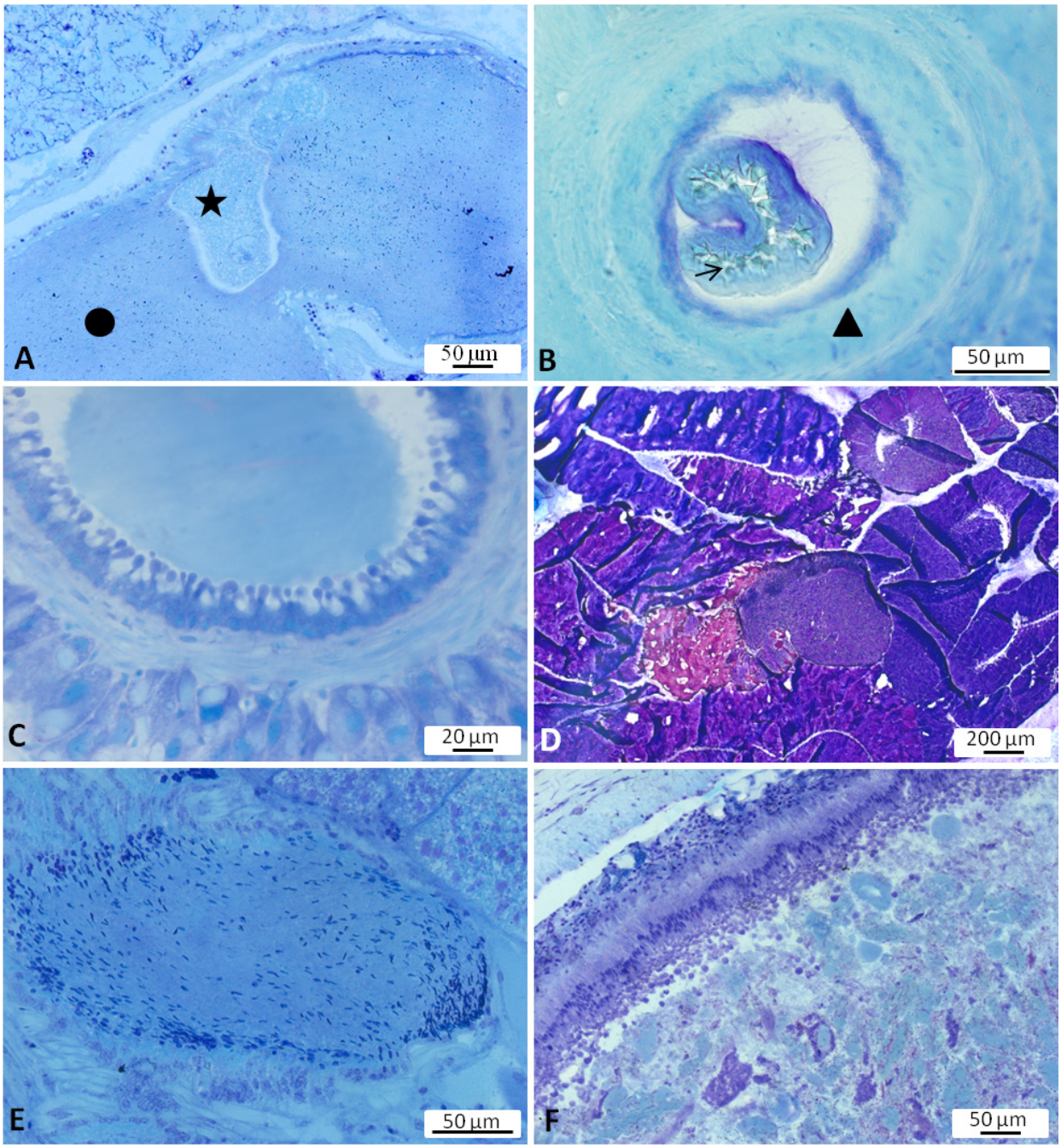

FIGURE 7. Histological sections of the genital system of *Peronia persiae* **sp. nov. A.** Ampulla. **B.** Cross section of penis. **C.** Penial accessory gland duct. **D.** Female gland mass (mucus gland and albumen gland). E. Receptaculum seminis. F. Spermatheca. Symbols: arrow=penial hook; asterisk=eggs; dot=sperms; head arrow=muscular layer.

Reproductive system. Hermaphrodite gland (gonad) compact, whitish with bright dots representing the oogonia; located slightly on the left side of the visceral cavity, next to the diaphragm. Hermaphroditic duct originating from gonad and continuing to a widened area, possibly the ampulla, containing sperm, as well as a few oocytes; duct with small epithelial cells (Figure 7A, Figure 9A). Vas deferens forming a long tube starting from hermaphroditic duct in the posterior third of body, running to the right side of the head, and ending in the penis; surrounded with a thick muscle layer and lined internally by ciliated cells. Prostate gland not observed. The anterior organs of male system consisting of the penial structure (penis with vas deferens and attached retractor muscle ending in penial sac) and penial accessory gland (glandular duct, hollow needle and end sac of penial gland). Both complexes opening into a common vestibule and sharing same male opening (Figure 9B). Penis usually inverted into the vas deferens (Figure 7B) ending in the penial sac. The outer epidermis of penis covered with spines. During evagination, the

usually outer layer turns to the outer side (Figure 8A). The hooks at the apical and distal areas are short and conical while the rest of spines in the middle part are long, sharp and curved downward at the tips. Fork-shaped spines existing in different areas of the penis (Figure 8B). Retractor muscle attached to the base of the penis in front part, running far into the posterior part of body. Penial accessory gland duct long, heavily coiled and swollen in posterior part, close to the opening; epithelium composed of columnar cells with apocrine secretion (Figure 7C). Light yellow needle apparatus straight, narrow and hollow; in one case tip slightly curved; length of needles around 1.3 mm (Figure 8C–D). The opening of needle usually blunt, only in one individual of sickle shape (Figure 8E). Sac-like structure without papillae. Proximal spermoviduct folded and embedded within the female gland mass. The latter composed of capsule gland (albumen gland), followed by membrane gland, leading into mucus glands (Figure 7D, Figure 9A). Membrane gland located ventrally underneath capsule gland, composed by columnar cells filled with reddish granules, mucus gland composed of columnar cells containing basal nucleus and many small, ovoid, violet staining granules. Presence of spiral glands could not be verified. Whitish receptaculum seminis composed of small epithelial cells, with attached sperm heads (allosperms) (Figure 7E). Brownish round to elongate spermatheca (or bursa copulatrix in the sense of Schmekel 1971) connecting to short distal oviduct; greenish staining contents present inside spermatheca; epithelium composed of apocrine secreting cells (Figure 7F). Female opening near the anus slightly to the right side.

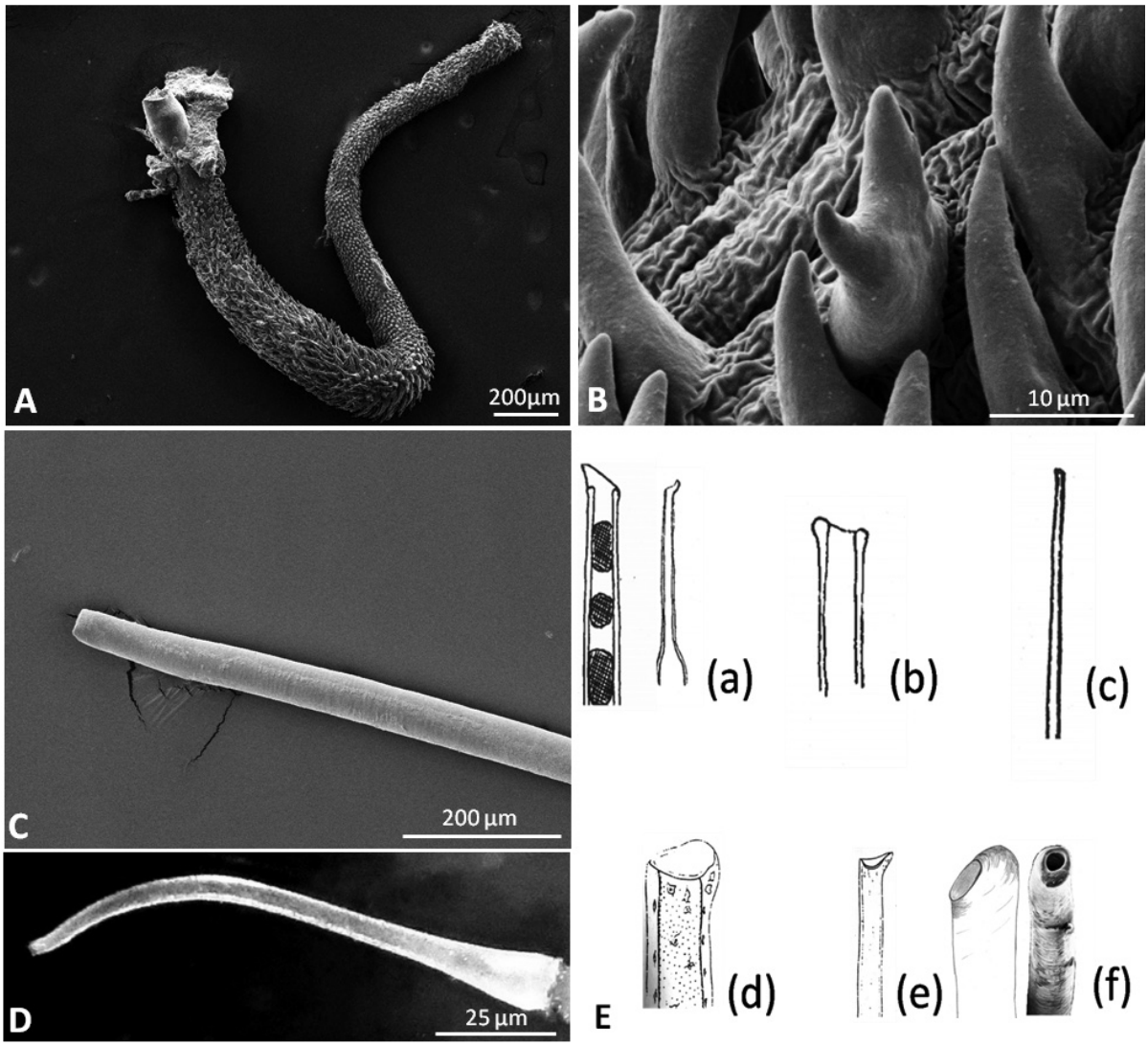

FIGURE 8. Cuticularized structures in the genital system of *Peronia persiae* **sp. nov. A–C.** Scanning electron microscopy. **A.** Partly everted penis. **B.** Forked penial hook. **C.** Typical needle in penial structure. **D.** Light microscopy. Curved needle, observed only once. **E.** Shape of needle aperture retrieved from literature and current study: (a) *P. gondwanae* (Labbé 1934); (b) *P. indica* (Labbé 1934); (c) *P. verruculata* (Awati & Karandikar 1948); (d) *P. verruculata* (Plate 1893); (e) *P. branchifera* (Plate 1893); *P. persiae* **sp. nov.** (drawing and SEM picture).

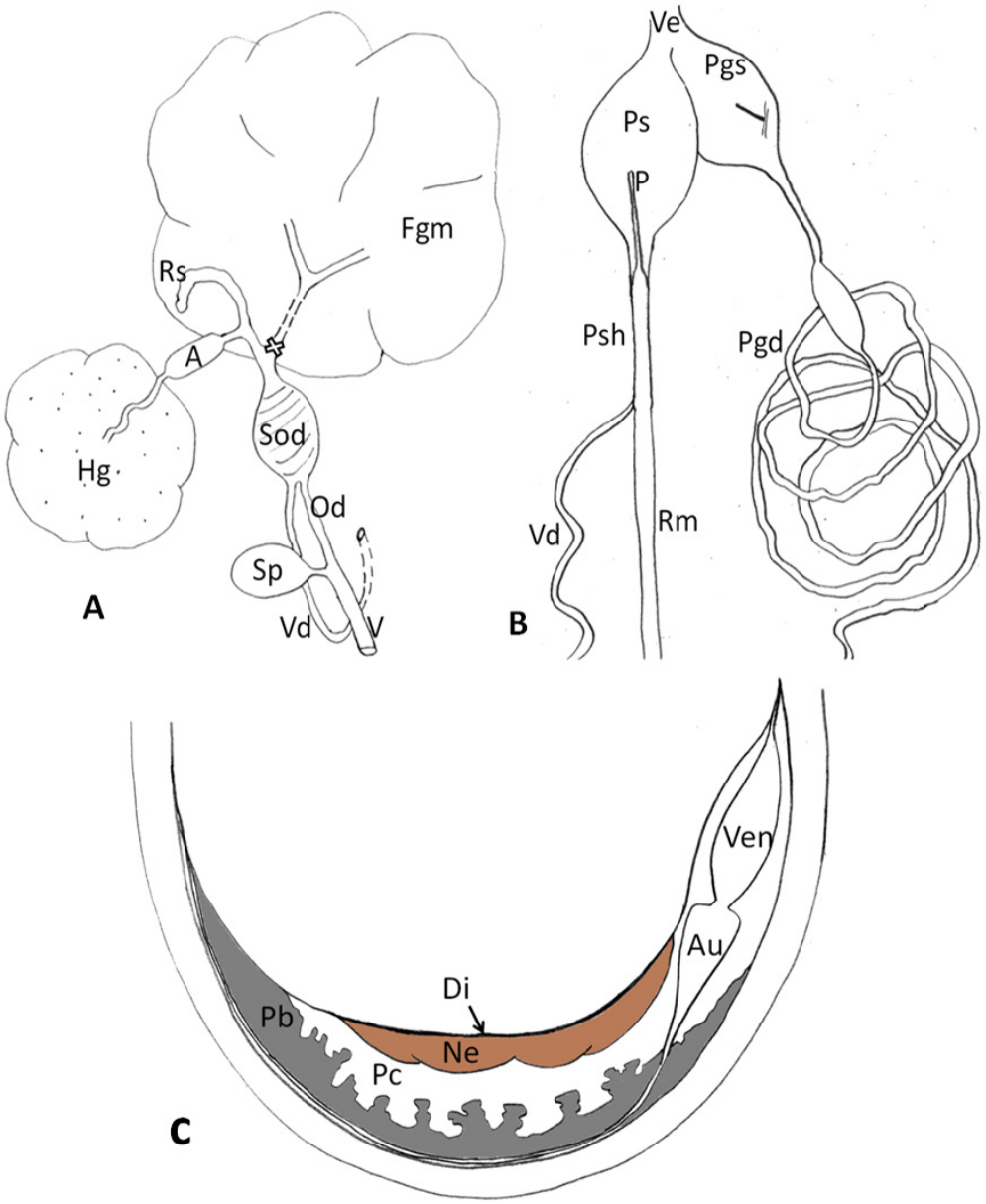

FIGURE 9. Reproductive and excretory system of *Peronia persiae* **sp. nov. A–B**. Female part of the hermaphroditic genital system. **B.** Male copulatory parts. **C.** Posterior part of the body opened and displayed from dorsal side. Abbreviations: A=ampulla; Au=auricle; Di=diaphragm; Fgm=female gland mass; Hg=hermaphrodite gland; Ne=nephridium; Od=oviduct; P=penis; Pb=pulmonary cavity branches; Pgd=penial gland duct; Pgs=penial gland sac containing needle; Ps=penial sac; Psh=penial sheath; Rm=retractor muscle; Rs=receptaculum seminis; Sod=spermoviduct; Sp=spermatheca; V=vagina; Vd=vas deferens (towards head); Ve=vestibule; Ven=ventricle. Connection from spermoviduct to female gland mass not clearly seen and indicated here with dotted line.

Circulatory and excretory system. Heart on the right side, divided into an anterior ventricle and posterior auricle within pericardial cavity. Nephridium posterior to the diaphragm. Extension of nephridium larger on the right side, than on the left side (Figure 11C).

Phylogeny. The phylogenetic analyses of the concatenated dataset (Figure 10) covering 11 of the 16 acknowledged genera and including 457 sequences, provide evidence of the monophyly of *Peronia* with high bootstrap support in the ML analyses (99) and close relationship with a clade formed by the monophyletic *Wallaconchis* (96), *Alionchis* (100) and *Paromoionchis* Dayrat & Goulding, 2019 (100). The close relationship of the four genera is supported by a bootstrap value of 97. One specimen retrieved from NCBI as *Scaphis* sp. groups within the genus *Peronia*. Further monophyletic genera are *Peronina* (100), *Melayonchis* (76), *Marmaronchis* (100), *Onchidina*

(100), and *Onchidella* (99). Monophyly of *Onchidium*, as shown in Dayrat *et al.* (2016) with a reduced dataset of Onchidiidae, cannot be seen in our tree, however misidentification of the paraphyletic genus *Platevindex* H. B. Baker, 1938 with members grouping with *Onchidium* cannot be excluded. In this overall analysis, *P. persiae* **sp. nov.** is clearly separate from all other *Peronia* species (Figure 10). These results are confirmed by analysing the reduced datasets including only *Peronia* sequences and running the analyses with COI only (Figure 11, Table 3), or the concatenated alignment with COI and 16S, and analyses of distance values (Figure 12). In these two analyses, the 11 specimens of the new species always group as a separate clade, usually as sister of *Peronia* sp. 3, and close to *Peronia* sp. from Singapore (COI, Figure 11) or close to *Peronia* sp. 4 and sp. 5 from Mozambique (concatenated data set, Figure 12).

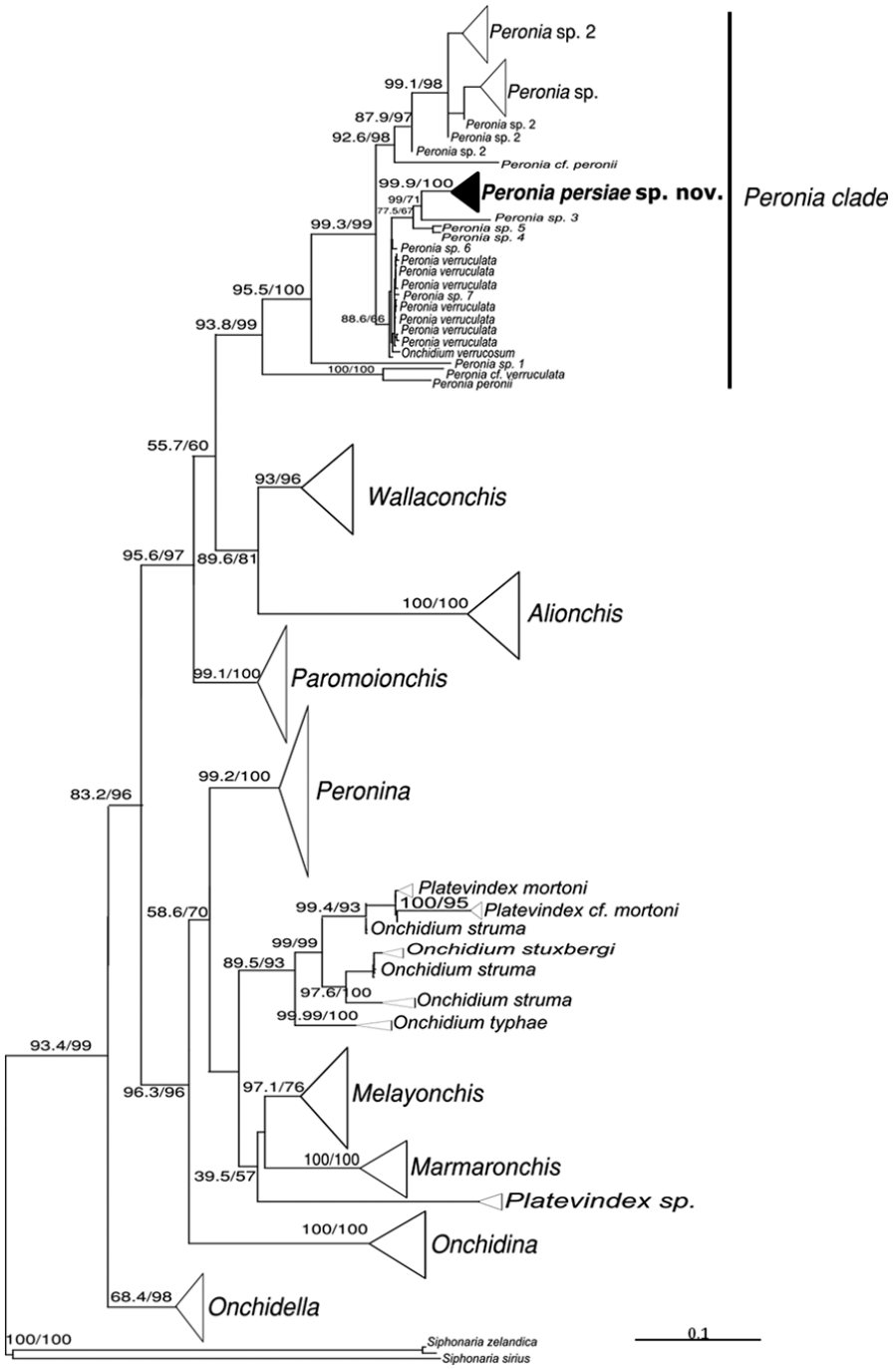

FIGURE 10. Phylogenetic reconstruction of Onchidiidae resulted from Maximum likelihood (ML) analysis of concatenated data set (COI and 16S) with *Siphonaria* as outgroup. *Peronia persiae* **sp. nov.** highlighted in bold. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap values respectively.

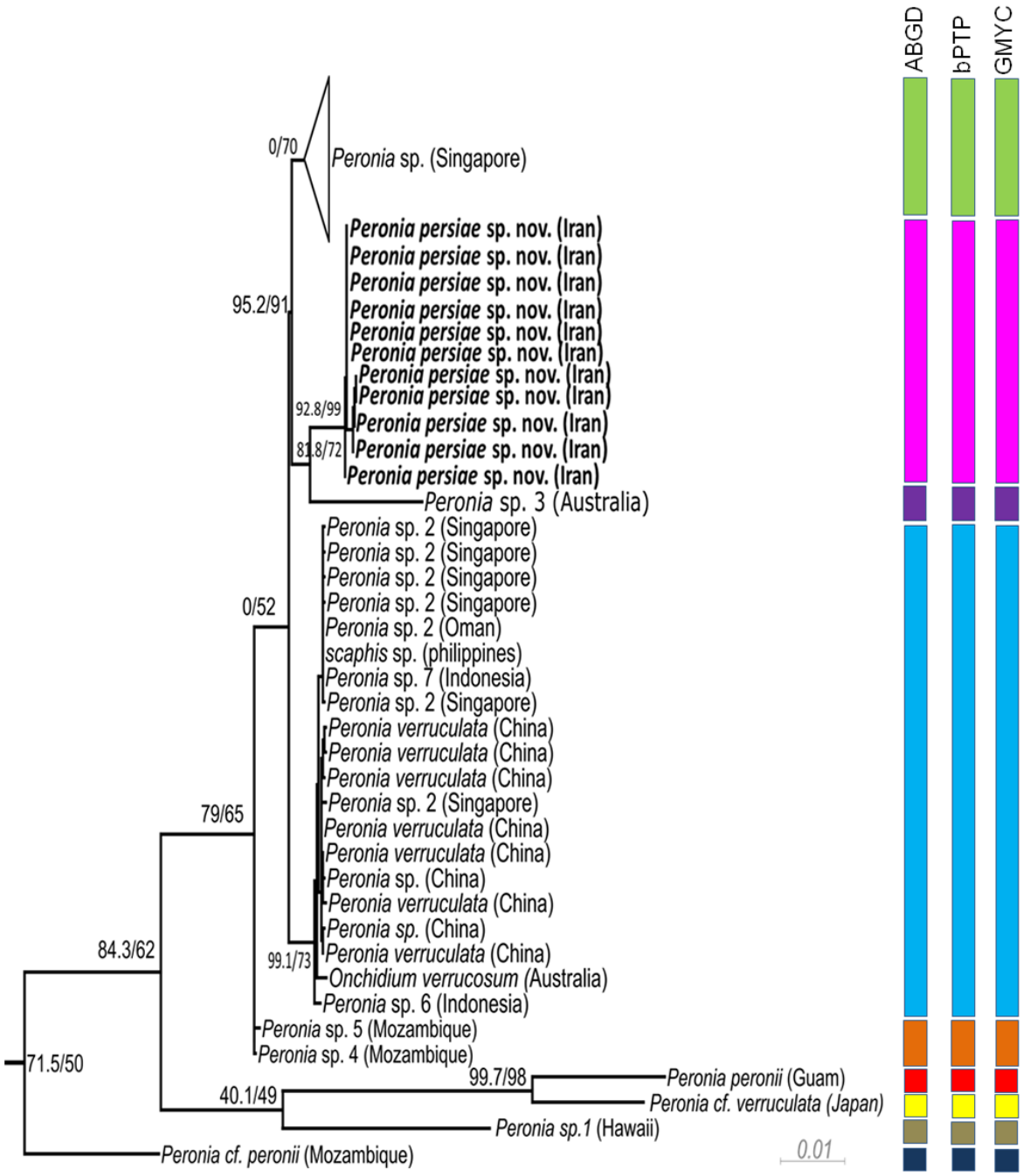

FIGURE 11. Maximum likelihood tree of the genus *Peronia* based on COI data set, *Wallaconchis graniferus* used as outgroup (not shown). Groups resulting from ABGD, GMYC, and bPTP tests indicated on the right side. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap values respectively.

Species delimitation. All three species delimitation methods applied to the COI (Figure 11) and 16S datasets indicate nine well supported groups in the genus *Peronia* (Figures 11–12, S1–S4). The Iranian specimens always form a separate taxon from *P. verruculata* specimens or any other *Peronia* species. The result of the ABGD test based on COI data shows a single and obvious barcode gap: intraspecific variability is less than 2% and interspecific variability more than 4%. The distance between the new Iranian and other close related species, *Peronia* sp. 4 and *Peronia* sp. 5, ranges between 7.0–8.0% (Table 3). All three tests applied on the separate two genes (Figure 12) show the same results: one Singapore group, assigned to *Peronia* sp., is indicated as a separate species. Another group, assigned to *Peronia* sp. 2 from Singapore and Oman, together with sequences assigned to *P. verruculata* and *Onchidium verrucosum* (misspelling of *P. verruculata*) from China and Australia, and a few sequences from differ-

ent localities (*Peronia* sp. from China, *Peronia* sp. 6 and *Peronia* sp. 7 from Indonesia, *Scaphis* sp. from Philippine Islands) are considered to be a single species. Results of GMYC and bPTP analysis, using the 16S data set, shows *Peronia* sp. 7 from Indonesia and *Scaphis* sp. as a separate group. *Peronia* sp. 4 and sp. 5 from Mozambique are indicated also as a single and distinct species; however, this last result was not retrieved in the GMYC and bPTP analysis using the 16S data set (Figure 12). *Peronia* sp. 3 from Australia and *Peronia* sp. 1 from Hawaii also are distinct species in all analyses. *Peronia* cf. *peronii* from Mozambique is considered as a distinct species, when applying only the CO1 dataset. This result is not confirmed in the GMYC and bPTP analysis using only the 16S data set. *P. peronii* from Guam (indicated as a separate species) clusters with a sequence of *Peronia* cf. *verruculata* from Japan (*Peronia* sp. according to Dayrat *et al.* 2016), which also forms a separate species (confirmed in all analyses).

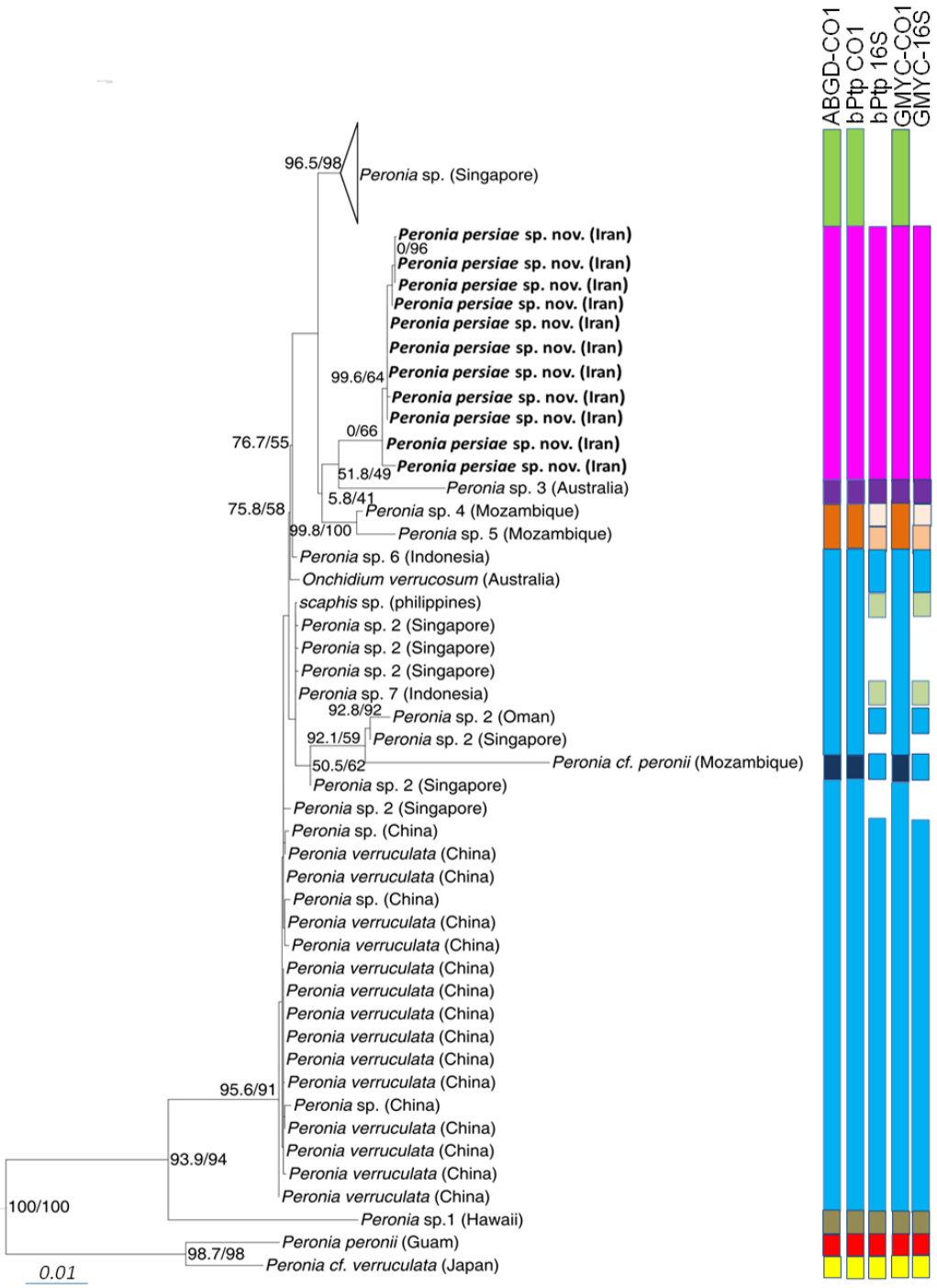

FIGURE 12. Maximum likelihood tree of the genus *Peronia* based on the concatenated alignment (COI and 16S). Groups resulting from ABGD, GMYC, and bPTP tests indicated on the right side. Numbers before and after slash indicate approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap values respectively.

TABLE 3. Intra- and interspecific pairwise genetic distances. Ranges from minimum to maximum distances are indicated (as percentages). * indicating a group of sequences comprising *Peronia verruculata* (China), *Onchidium verrucosum* (Australia), *Peronia* sp.2 (Oman), *P*. sp.6 (Indonesia), *P*. sp.7 (Indonesia), *P*. sp (China), *Scaphis* sp. (Philippine Islands), and *Peronia* sp.2 (Singapore).

	Peronia sp. Clade (Singapore)	*Peronia verruculata* group*	*Peronia* sp. 4, sp. 5 (Mozambique)	*Peronia persiae* **sp. nov.** (Iran)	*Peronia* sp.3 (Australia)	*Peronia* cf. *peronii* (Mozambique)	*Peronia* sp.1 (Hawaii)	*Peronia peronii* (Guam)	*Peronia* cf. *verruculata* (Japan)
Peronia sp. Clade (Singapore)	0–9								
Peronia verruculata group*	4–6	0–2							
Peronia sp. 4, sp. 5 (Mozambique)	5–7	6–9	0–1						
Peronia persiae **sp. nov.** (Iran)	6–9	8–12	7–8	0–1					
Peronia sp.3 (Australia)	10–11	10–12	10–11	10–11	0				
Peronia cf. *peronii* (Mozambique)	15	15–17	14–15	14–15	16.40	0			
Peronia sp.1 (Hawaii)	17–18	15–18	18–19	17	16.70	20.90	0		
Peronia peronii (Guam)	19–20	20–22	19	21	21.20	18.90	21.40	0	
Peronia cf. *verruculata* (Japan)	20	20–21	19	20	21.00	19.31	18.90	13.50	0

Discussion

Morphology. Although several species of *Peronia* have been described, most information is available only for *P. verruculata* and *P. peronii*. Nevertheless, *P. persiae* **sp. nov.** can be distinguished from all described species by several features (see Table S1) and resembles most *P. verruculata*. Our new species has branchial gills covering only the posterior part of the body (Figure 2B) as is described for many *Peronia* species (Farran 1905; Britton 1984). Only *P. peronii* has gills scattered all over the notum (Plate 1893; Labbé 1934; Solem 1959). Similar to *P. verruculata*, *P. persiae* **sp. nov.** lacks spicules in the notum (Awati & Karandikar 1948). Information about this character is missing for *Peronia anomala* Labbé, 1934, *Peronia lata* Labbé, 1934 and *Peronia branchifera* Plate, 1893; however, all other described *Peronia* species possess spicules. The visceral cavity in *P. persiae* **sp. nov.** is strongly pigmented like in *P. verruculata* (Awati & Karandikar 1948), while the visceral cavity of *P. peronii* is not pigmented (Labbé 1934; Plate 1893). Various photoreceptive systems are thoroughly described for *P. verruculata* (Katagiri *et al.* 1985) including stalk eyes, dorsal eyes, free dermal photoreceptor cells in the notum, and photosensitive neurons in the central nervous system. At least the first two types of photoreceptors are present in all *Peronia* species (Table S1). One dorsal tubercle has several eyes and these tubercles are scattered over the dorsal notum. Only *P. lata* has one dorsal eye per tubercle and thus differs from our new species.

P. persiae **sp. nov.** lacks the pharyngeal wall teeth, which are present in *P. verruculata* (Awati & Karandikar 1948). Additionally, the new species has a larger radula with more rows and more teeth per row, when comparing *P. verruculata* specimens of same size with our specimens (both 65 mm): 71×87.1.87 versus 65×66.1.66 (Awati

& Karandikar 1948). The lateral cusps on both sides of the rachidian teeth are more elongate and pointed, and the main cusp is much more developed in *P. persiae* **sp. nov.** than in *P. verruculata* (Plate 1893; Awati & Karandikar 1948; Britton 1983), thus similar to *P. peronii* (Labbé 1934). The concave internal part of the lateral teeth was not mentioned before (Figure 5C) and might be a special character of the new species. Most onchidiid species have a stomach divided into four parts (Weiss & Wägele 1998; Dayrat *et al.* 2016). However, stomachs with three or two parts are also reported (Dayrat 2010a; Dayrat 2010b). Awati & Karandikar (1948) considered the third and fourth parts as one single chamber in *P. verruculata*. The stomach of the here investigated *P. persiae* **sp. nov.** has four parts in which the fourth has a ciliated epithelium. The intestine with two loops can be assigned to type II. Descriptions for *P. peronii* and *P. verruculata* vary between the more common intestinal type I (with one loop) and type II (Plate 1893; Labbé 1934; Marcus & Marcus 1959).

The position, symmetric or asymmetric, of the nephridium is mentioned as a distinguishing feature for *Peronia* species. *P. persiae* **sp. nov.** has an asymmetric nephridium with the right side larger than the left side. According to the illustration of *P. verruculata* (Awati & Karandikar 1948, Figure 38) the nephridium is larger on the left side. However, Labbé (1934) mentioned a symmetric nephridium for *P. verruculata*, as well as for *P. peronii.* These differences in the description show that the nephridium can vary and therefore cannot be considered as a diagnostic character.

In many heterobranch species, a part of the hermaphroditic duct is swollen into an ampulla, in which autosperm is stored before release during copulation. An ampulla is not described for any Onchidiidae species except a specimen investigated by Marcus (1971) and assigned to *Onchidium simrothi* Plate, 1893 (Marcus 1971). This specimen was recently assigned to the new genus *Wallaconchis*: Goulding *et al.* (2018b) re-examined the type material of *O. simrothi* and subsequently considered this name as a *nomen dubium*. They did not mention an ampulla for any *Wallaconchis* species. It seems that this character is difficult to find in onchidiids, or it is only present in a few species. The feature that we describe here in the gonoduct, filled with irregularly lying sperm, is very similar to the structure that is described by Schmekel (1971) as ampulla in Nudibranchia. We herein report for the first time the existence of ampulla in *Peronia*.

The vas deferens often exhibits an enlarged special area (considered as prostate) with glandular cells producing prostatic material (e.g., Wägele & Willan 2000). In histological investigations, prostatic cells are visible by their typical glandular cells. Interestingly, no prostate was detected in *P. persiae* **sp. nov.**, while Awati & Karandikar (1948) described it in detail for *P. verruculata*. Unfortunately, no further information about the prostate is available for any other *Peronia* species. The penial complex usually has the same structures in most Onchidiidae species, including members of *Peronia*. The epidermis of the penis is usually covered by spines. We observed some differences in *P. persiae* **sp. nov.** such as the penial hooks in the anterior and posterior part of the penis being smaller than those of the middle region. Short spines in the posterior part are not described in *P. verruculata*. Additionally, some hooks are fork-shaped, and these special forms are scattered all around the penis. The fork-shaped hooks are not known for any other *Peronia* or onchidiid species. Another distinguishable character in *P. persiae* **sp. nov.** is the absence of calcareous granules which were described on the hooks of the anterior part of the penis in *P. verruculata* (Awati & Karandikar 1948; Britton 1984).

The penial gland duct of a member of Onchidiidae is investigated histologically here for the first time. Its function might be provision of substances to help in sperm transfer. No papillae of the penial gland have been observed in *P. persiae* **sp. nov.**, while this structure is described for *P. verruculata* (Britton 1984) and *Peronia gaimardi* Labbé, 1934 (Labbé 1934). *Peronia ferruginea* Lesson, 1831 completely lacks the penial gland (Lesson 1830, Britton 1984). The colour of the penial needle in *P. persiae* **sp. nov.** is light yellow, like in *P. verruculata* (Awati & Karandikar 1948), but dark brown in *P. peronii* (Plate 1984). The shape of the penial needle opening can vary among the *Peronia* species (Figure 8E). It is shovel-shaped in *P. branchifera*, oblique or shovel shaped in *Peronia gondwana* Labbé, 1934, oblique in *P. indica*, and unilateral thickened in *P. verruculata*. *P. persiae* **sp. nov.** has two new different shapes of needle openings, which are not described from any other *Peronia* species. In most individuals of *P. persiae* **sp. nov.** it is blunt and only in one individual it is sickle shaped. All other parts of the genital systems are similar to the ones described for *P. verruculata* and *P. peronii*.

Based on these combinatory anatomical differences (Table S1), we cannot assign our specimens to any described *Peronia* species and therefore consider it as a distinct new species.

Molecular data. This first molecular analysis of the Onchidiidae including all available sequences and covering 11 genera, provide good evidence for the monophyly of *Peronia* and nearly all other onchidiid genera. *Platev-*

index and *Onchidium* are not monophyletic, thus supporting former analyses, based on smaller data sets without the five recently described genera *Wallaconchis*, *Alionchis*, *Paromoionchis*, *Melayonchis* and *Marmaronchis* (Dayrat *et al*. 2017, 2018, 2019; Goulding *et al*. 2018a; Goulding *et al*. 2018b). Species delimitation tests clearly show that *P. persiae* **sp. nov.** is a distinct species. It does not group with any of the other specimens assigned to *P. verruculata* or other, unidentified *Peronia* sequences. It thus supports the results based on the morphological analysis. Additionally, our results indicate the presence of several further unidentified and potentially undescribed species of *Peronia*. Different species delimitation analyses (GMYC, ABGD, bPTP) based on COI and 16S genes, detected nine species within this genus. It is not clear whether these groups represent already described species, because no sequences are available for many recognized *Peronia* species and therefore a revision of the genus is highly needed.

Conclusions

Integrative species delimitation analyses are well-established in heterobranchs (e.g. Jörger *et al.* 2012, Krug *et al.* 2013; Padula *et al.* 2014). Investigating morphology with different methodologies and combining these results with those obtained from an array of suitable molecular approaches can provide good evidence in species delimitation and descriptions. Our analysis has shown for the first time the presence of a new *Peronia* species in the intertidal flats along the southern coast of Iran. With this new species, ten *Peronia* species are considered as valid. Future analyses will show whether any of the sequences in our analysis confirmed here to belong to the genus *Peronia* can be assigned to one of the former described species. Whether *P. persiae* **sp. nov.** is endemic to Iran or has a wider geographic distribution is not known, more studies in the Persian Gulf and adjacent areas are necessary. The lack of records of other onchidiid species in this region suggests that *P. persiae* **sp. nov.** may have evolved certain adaptations to live in the extreme environmental conditions of the intertidal flats of Iran.

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TABLE S1. Features of valid species within the genus *Peronia*. Abbreviation in brackets indicates authors and year of publication: A=Awati & Karandikar 1948, At=Attaran *et al*. 2015, B=Britton 1984, Bi=Bitaab *et al*. 2015, Br=Bretnall 1919, C= Cuvier 1804, F=Farran 1905, H=Hyman 1999, L=Labbé 1934, Le=Lesson 1830, M=Marcus & Marcus 1959, Pa=Patil & Kulkarni 2013, P=Plate 1893, S=Solem 1959, W=White 1951.

Species	*P. anomala*	*P. branchifera*	*P. gaimardi*	*P. gondwanae*	*P. indica*	*P. lata*	*P. ferruginea*	*P. peronii*	*P. verruculata*	*Peronia persiae* sp. nov.
Original name	*Peronia anomala* Labbé, 1934	*Oncidium branchiferum* Plate, 1893	*Peronia gaimardi* Labbé, 1934	*Paraperonia gondwanae* Labbé, 1934	*Quoyella indica* Labbé, 1934	*Scaphis lata* Labbé, 1934	*Onchidium ferrugineum* Lesson, 1831	*Onchidium peronii* Cuvier, 1804	*Onchidium verruculatum* Cuvier, 1830	-
Maximum length of body (mm)	10 (L)	27.5 (P), 30 (L)	84 (L)	32 (L)	22 (L)	28 (L)	30 (L), 38 (Le)	155 (M),122 (L),130 (C), 53 (W), 155 (M), 104 (Br), 139.7 (P), 100 (At), 20 (Pa), 85 (Bi)	31 (Br), 40 (B), 50 (P), 69 (A), 70 (Pa)	65
Dorsal eye	-	8 tubercles with eyes (P), 2-4 eyes per tubercle (L & P)	-	3-4 eyes per tubercle (L)	3-4 eyes per tubercle (L)	single eye at each tubercle (L)	2-3 or 4 eyes per tubercle (L)	4-5 eyes per tubercle (P)	6 tubercles with 2-5 eyes (F & L), yellow tipped papilla bearing 3-4 black eyes (H), 1-4 eyes per tubercle (Br), single or 1-6 eyes per tubercle (A), 6-7 eyes per tubercle (P)	maximum 16 tubercles, 2-4 eyes per tubercle
Integument	thin (L)	-	thick, large spicules widely spaced (L)	thick, with large spicules (L)	with spicules (L)		thin, with large spicules (L), very thick and fleshy (Le)	with irregular spicules (L)	thick, without spicules (A)	thick, without spicules
Type of intestine	type II (L)	type I (P & L)	type I (L)	type V, sometimes type I (L)	type V (L)	type I (L)	type I (L)	type I or II (M), type I (L), type I (P)	type I or rarely type II (L), type II (H), type I, occasionally type II (Br), type I (P), type I (A)	type II

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TABLE S1. (Continued)

Species	*P. anomala*	*P. branchifera*	*P. gaimardi*	*P. gondwanae*	*P. indica*	*P. lata*	*P. ferruginea*	*P. peronii*	*P. verruculata*	*Peronia persiae* sp. nov.
Colour of notum and hyponotum	-	middle of notum yellow with black brown patches, outer part black brown (P), dirty yellow notum (L)	dirty yellow with black spots (L)	greyish yellow (L)	-	uniform greenish yellow (L)	notum light yellow (L), notum intense ferruginous red (Le), yellowish white with black ventral head (L & Le)	very variable: greyish or blackish yellow or marble with spots (L), greenish or blackish, hyponotum pale yellow (C), green grey with light patches (M), olive (Br), black to light brown (P), dark green to brown with grey patches (At)	yellow greyish with yellow or brown spot, hyponotum yellowish white (L), olive with brown pattern, hyponotum light olive (Br), brown with uniform red-brown or reddish markings and yellow to brown notum margin, hyponotum bluish grey (H), grey to blue grey with stripes or black mottling (Br), grey yellow with black brown patches(P), brown (Pa)	notum muddy green to grey, hyponotum yellow green
Branchial gill position and form	posterior 1/4 (L)	posterior 1/6 (P), long finger shape extensions (L & P)	-	Posterior (L)	posterior 1/6 (L)	Posterior (L)	posterior 1/6 (L & Le), short tubercles, gathered in small bundles of five to six (Le)	all over the notum (L, P & S), spongy appearance (C), close to posterior mantel border (Br)	10 gills in posterior 1/4-1/5 (F & L), short gills at posterior side (Br), posterior 1/4, short and finger shape (P)	15 branchial gills, posterior 1/6, very short, branched from the base
Male pore	-	one pore on left side of right tentacle (P)	one pore on left side of right tentacle (L)	-	two separate pores (L)	-	-	-	one pore on left side of right tentacle (A)	one on left side of right tentacle

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TABLE S1. (Continued)

Species	*P. anomala*	*P. branchifera*	*P. gaimardi*	*P. gondwanae*	*P. indica*	*P. lata*	*P. ferruginea*	*P. peronii*	*P. verruculata*	*Peronia persiae* sp. nov.
Shape of aperture of needle and colour	-	shovel-shaped (P)	-	unilateral thickening (L), needle narrow	oblique (L)	-	-	needle black brown (P)	unilateral thickening (P), needle yellow and very narrow (A)	Blunt and only one sickle shaped, needle light yellow
Papillae at base of penial structure	-	absent (P)	present (L)	-	-	-	present (L), absent (B)	-	present (Br)	absent
Penial hooks of penis	-	-	-	-	-	-	-	-	anterior part with hooks, posterior part without hooks (Br), anterior and posterior parts with hooks, but posterior with large spines and granules (A), without "chondroid" elements (P)	posterior spines much larger than anterior and both parts without calcareous granules
Prostate	-	-	-	-	-	-	-	-	Unidentified structure (no name provided) depicted on top of nidamental glands (P), Present (A), present (H)	Absent
Spermatheca (receptacle seminalis)	-	large (P)	-	-	-	-	-	large,12-15 mm (L), much larger than receptaculum (M), larger than receptaculum(P)	Large (H), short and stalked (P), round (A)	Roundish to elongate and short stalked
Receptaculum seminis (vesicule seminale)	-	Conical (P)	-	-	-	-	-	large,12-15 mm (L), blind sac (M), large with thin stripe (P)	large, tubular structure, 10mm (L), small and smooth (H), large, tubular (P), finger shaped (A)	present

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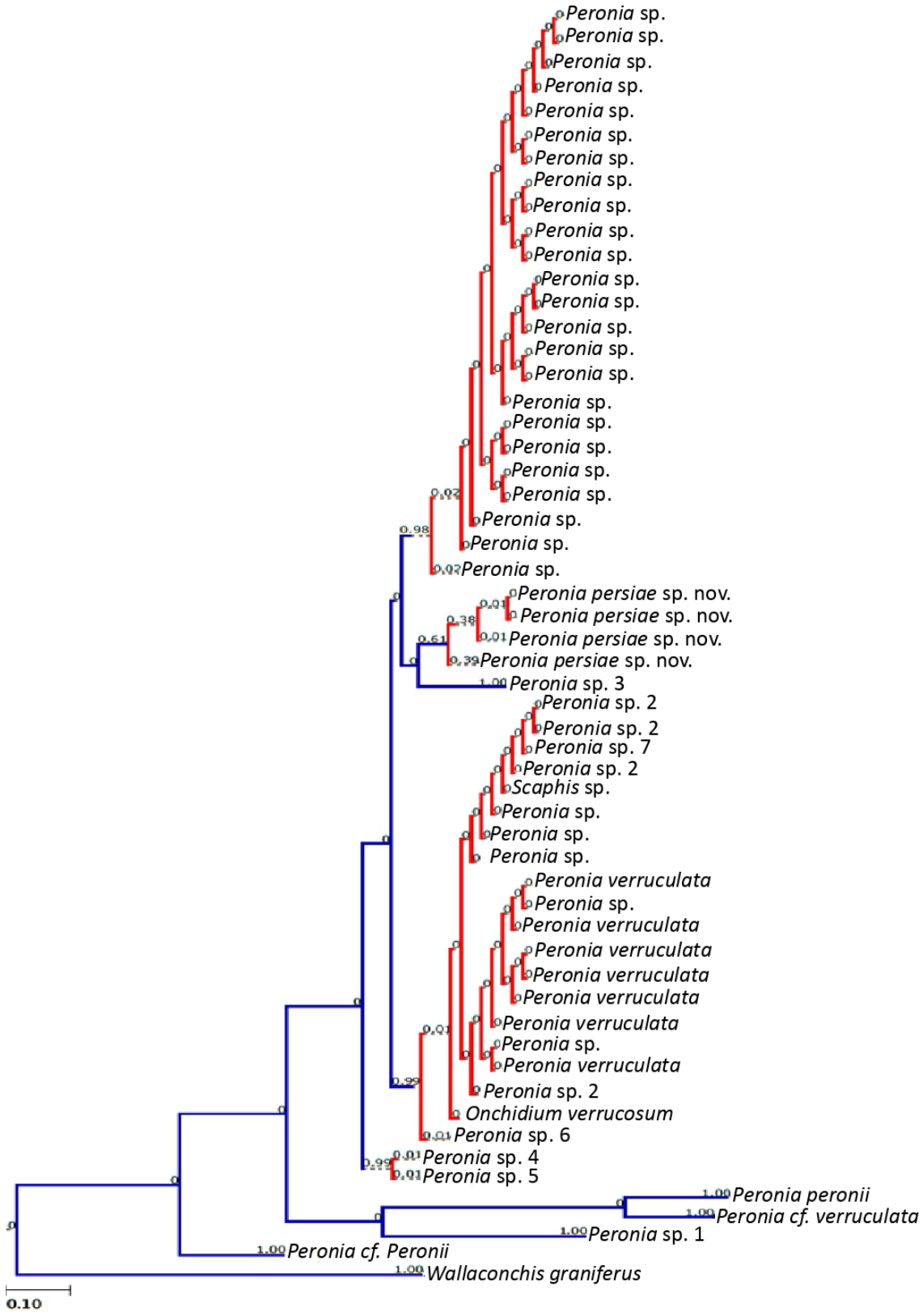
TABLE S1. (Continued)

Species	*P. anomala*	*P. branchifera*	*P. gaimardi*	*P. gondwanae*	*P. indica*	*P. lata*	*P. ferruginea*	*P. peronii*	*P. verruculata*	*Peronia persiae* sp. nov.
Position of respiratory opening in posterior part	-	median (P), median (L)	median (L)	-	to the right side (L)	to the right side (L)	-.	median or a little bit to the right side (L), median or slightly to the right side (M), median (P)	median (L), median (H), median (P)	median
Visceral cavity	slightly pigmented (L)	pigmented (L)	non or partially pigmented (L)	-	light brown pigmented (L)	non pigmented (L)	non pigmented or light brown or weakly black pigmented (L)	non pigmented or scattered pigmentation (L & P)	strongly pigmented (L), pigmented from light to pronounced colour (Br)	strongly black pigmented
Rectal gland	-	absent (P)		-	-	-	present (B)	-	absent (P)	absent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Peronia sp.
Peronia sp.
Peronia sp.
Peronia sp.
Peronia sp.
Peronia persiae sp. nov.
Peronia persiae sp. nov.
Peronia persiae sp. nov.
Peronia persiae sp. nov.
Peronia sp. 3
Peronia sp. 2
Peronia sp. 2
Peronia sp. 7
Peronia sp. 2
Scaphis sp.
Peronia sp.
Peronia sp.
Peronia sp.
Peronia verruculata
Peronia sp.
Peronia verruculata
Peronia verruculata
Peronia verruculata
Peronia verruculata
Peronia verruculata
Peronia sp.
Peronia verruculata
Peronia sp. 2
Onchidium verrucosum
Peronia sp. 6
Peronia sp. 4
Peronia sp. 5
Peronia peronii
Peronia cf. verruculata
Peronia sp. 1
Peronia cf. Peronii
Wallaconchis graniferus
0.10

FIGURE S1. Rooted ultrametric tree from Bayesian analysis with branches coloured according to the bPTP species delimitation analysis for the genus *Peronia* based on COI alignment. Bayesian support values are shown above the branches. Blue lines indicate branching processes among species, red lines indicate branching processes within species. *Wallaconchis graniferus* as outgroup and identical sequences are removed.

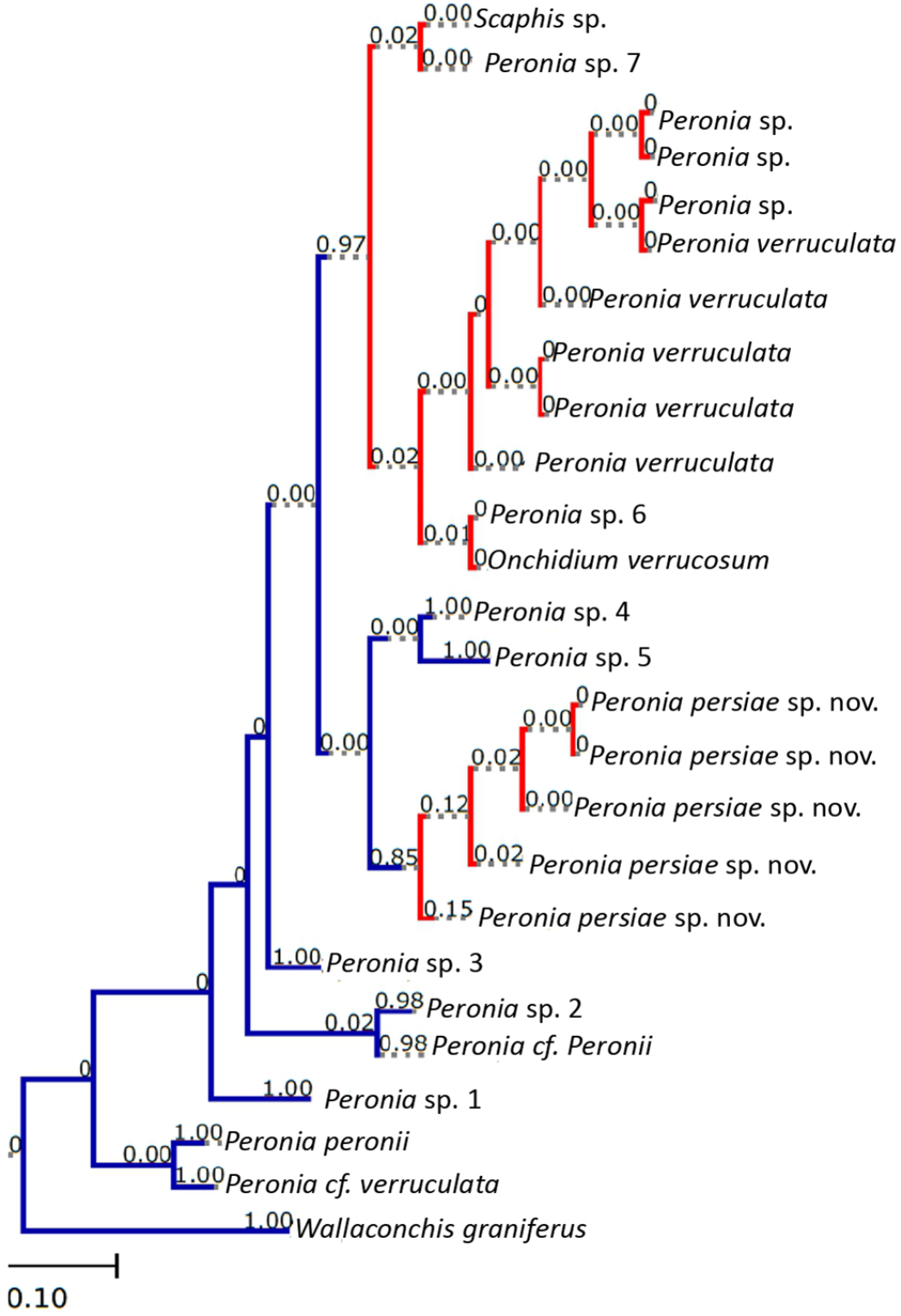

FIGURE S2. Rooted ultrametric tree from Bayesian analysis with branches coloured according to the bPTP species delimitation analysis for the genus *Peronia* based on the 16S alignment. Bayesian support values are shown above the branches. Blue lines indicate branching processes among species, red lines indicate branching processes within species. *Wallaconchis graniferus* as outgroup and identical sequences are removed.

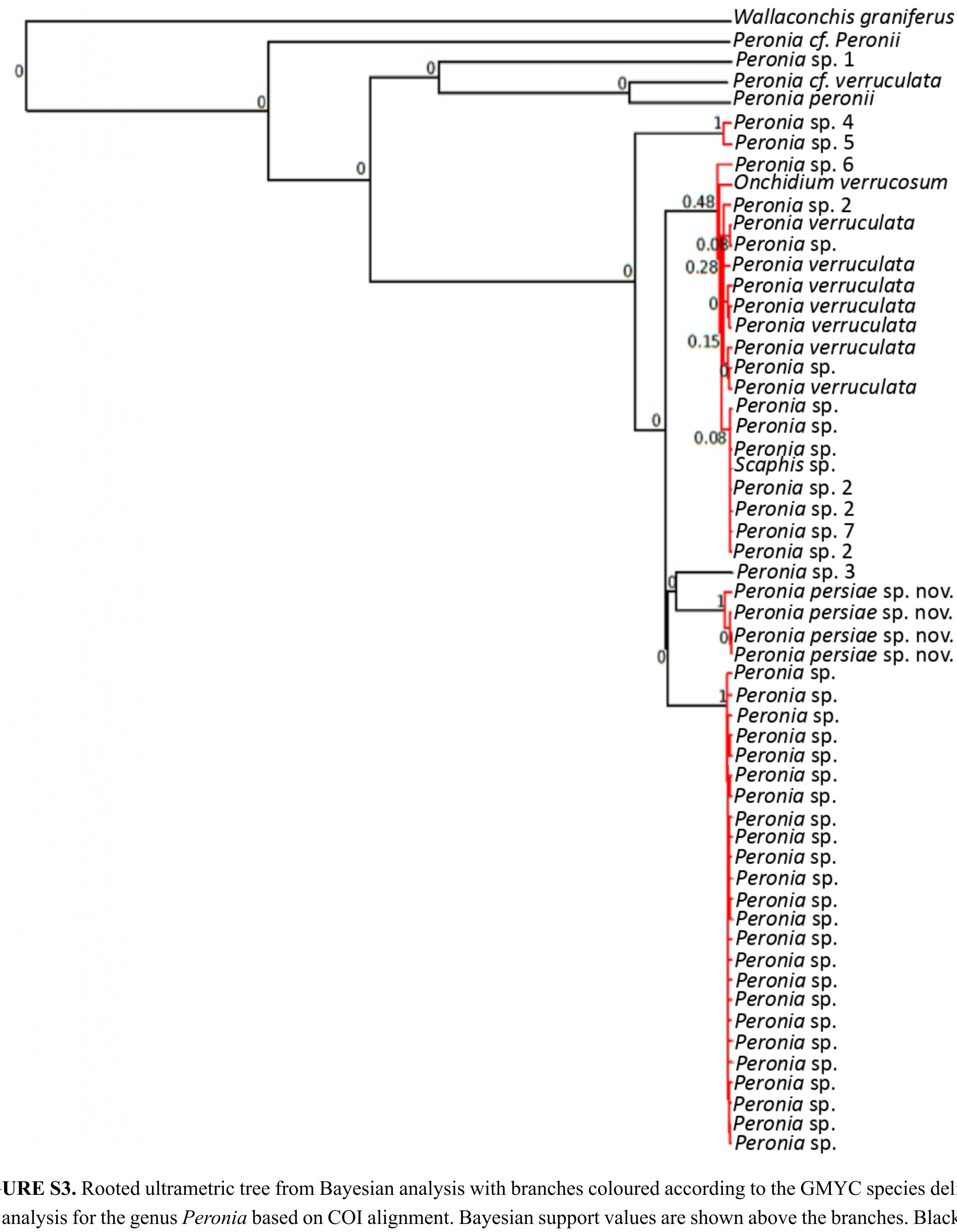

FIGURE S3. Rooted ultrametric tree from Bayesian analysis with branches coloured according to the GMYC species delimitation analysis for the genus *Peronia* based on COI alignment. Bayesian support values are shown above the branches. Black lines indicate branching processes among species, red lines indicate branching processes within species. *Wallaconchis graniferus* as outgroup and identical sequences are removed.

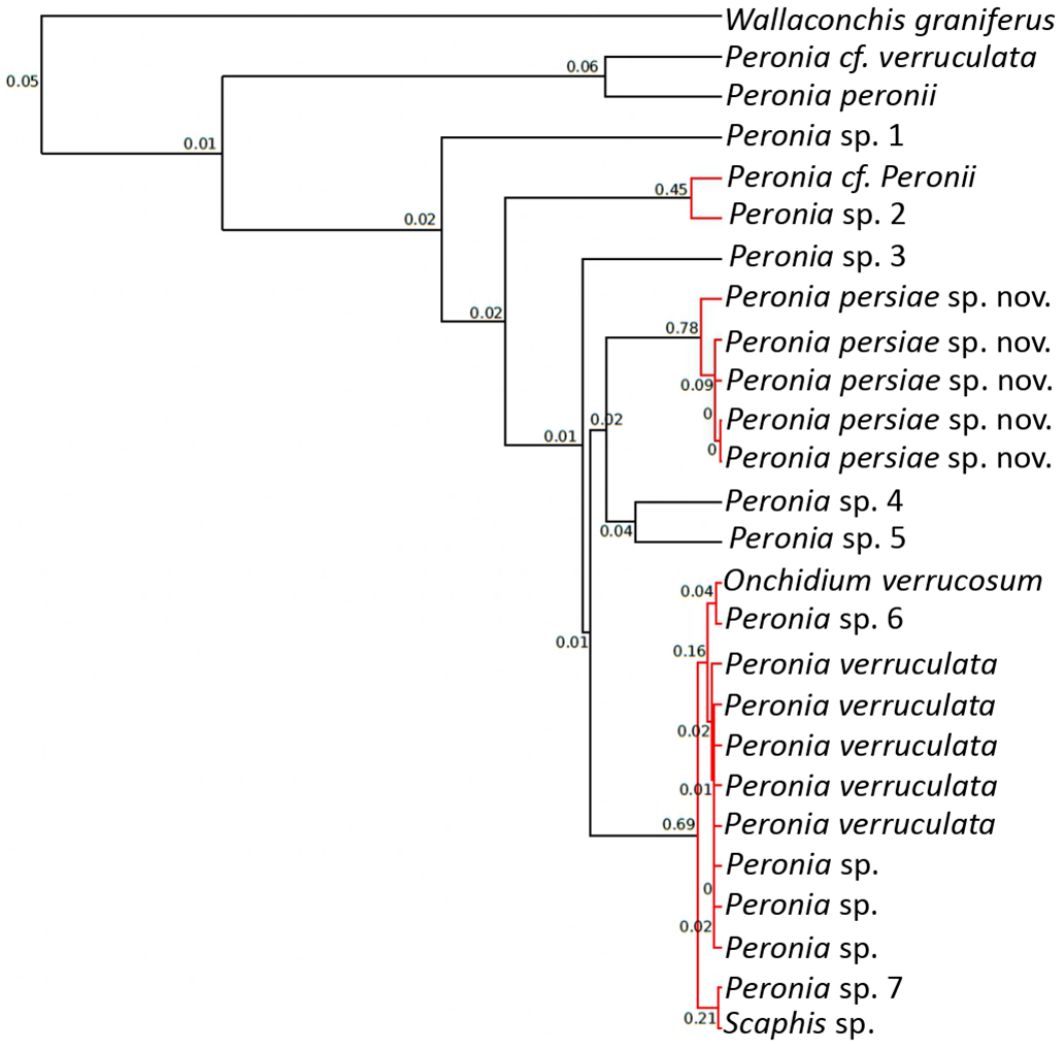

FIGURE S4. Rooted ultrametric tree from Bayesian analysis with branches coloured according to the GMYC species delimitation analysis for the genus *Peronia* based on 16S alignment. Bayesian support values are shown above the branches. Black lines indicate branching processes among species, red lines indicate branching processes within species. *Wallaconchis graniferus* as outgroup and identical sequences are removed.