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Unresolved taxonomy confounds invasive species identification: the *Lysmata vittata* Stimpson, 1860 (Decapoda: Caridea: Lysmatidae) species complex and recent introduction of *Lysmata vittata* sensu stricto in the western Atlantic

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ABSTRACT

Peppermint shrimp resembling *Lysmata vittata* Stimpson, 1860, a species native to the Indo-West Pacific, were found in the lower Chesapeake Bay and adjacent coastal embayments in 2013, representing the first recorded introduction of this species in the northwestern Atlantic. Conflicting morphological descriptions, inconsistent morphological terminology, and limited molecular data (i.e., unresolved taxonomy), as well as the destruction of the type material of *L. vittata*, created uncertainty regarding proper identification. We provide the first phylogeny incorporating individuals from across the presumed native and introduced range of *L. vittata*. Morphological and phylogenetic analyses clearly indicate *L. vittata* represents a species complex of two widely divergent groups: 1) "Bruce Type" with a uniramous dorsal antennule that agrees with A.J. Bruce's 1990 redescription of *L. vittata*, and 2) "Rauli Type" with a one-article accessory branch on the dorsal antennule that agrees most closely with the junior synonym *L. rauli* Laubenheimer & Rhyne, 2010. Given the taxonomic ambiguity surrounding *L. vittata*, we designate the individual used by A.J. Bruce to redescribe *L. vittata* and incorporated in our analyses as a neotype to fix the identity of this species. We therefore identify introduced North American and New Zealand populations as *L. vittata* sensu stricto and postulate that the native range spans temperate/subtropical East Asia. These data suggest that *L. rauli* is a valid species, which includes a possible undescribed sister species. We confirm the presence of *L. californica* Stimpson, 1866 in New Zealand, the first non-native record for this species. We also provide data suggesting *L. dispar* Hayashi, 2007 may be more widespread in the Indo-West Pacific than currently known and consider *L. lipkei* Okuno & Fiedler, 2010 to be a likely junior synonym.

Key Words: 16S rRNA, accessory branch, fish-cleaning shrimps, genetic barcoding, non-native species, *Lysmata californica*, *Lysmata dispar*, *Lysmata rauli*, peppermint shrimp, species delimitation

INTRODUCTION

Non-indigenous species pose a significant threat to global biodiversity, populations of native species, ecosystem function, economic stability, and human health (Bax *et al.*, 2003; Molnar *et al.*, 2008; Ruiz *et al.*, 1997; Dueñas *et al.*, 2018). Identification of invasive taxa and the subsequent determination of their native ranges and habitat preferences are critical in predicting potential ecological and economic impacts and developing biocontrol and mitigation strategies (Kumschick *et al.*, 2015; McLeish *et al.*, 2020). These efforts are often complicated by uncertainty surrounding the systematics and ecology of introduced and native taxa (Albano *et al.*, 2021). Many marine and aquatic invaders may belong to species complexes, which can contain more than one morphologically similar species, each with its own environmental requirements and invasion potential (Filipová *et al.*, 2010; Zhan *et al.*, 2010). The accurate identification of native, invasive, and cryptogenic species can be hampered by a lack of rigorous taxonomic investigations and/or conflicting taxonomic and morphological assessments (i.e., unresolved taxonomy; Carlton, 2009; Hirsch *et al.*, 2017; Marchini & Cardeccia, 2017).

The caridean shrimp genus *Lysmata* Risso, 1816 has received much attention from taxonomists in recent years with nearly half of the currently recognized species ($N = 48$) described in the last two decades (WoRMS, 2021a); however, the systematics of this genus remains unsettled (Baeza, 2010; Fiedler *et al.*, 2010; Soledade *et al.*, 2013; Pachelle *et al.*, 2016; Alves *et al.*, 2018; González-Ortegón *et al.*, 2020). *Lysmata* was split into two genera: *Hippolysmata* Stimpson, 1860 and *Lysmata*, the latter characterized by a multi-article accessory branch on the dorsal antennular flagellum (hereafter referred to as the accessory branch) and absent in the former (Stimpson, 1860; Chace, 1972). Several species were nevertheless misclassified upon initial description as *Hippolysmata*, yet they possessed a multi-article accessory branch, including *L. intermedia* Kingsley, 1878, *L. moorei* Rathbun, 1901, *L. ternatensis* De Man, 1902, and *L. trisetacea* Heller, 1861. Kubo (1951) and Chace (1972) both placed *Hippolysmata* in synonymy with *Lysmata* due to inter- and intraspecific variability in the structure of the accessory branch, particularly in relation to the type species of *Hippolysmata*, *Lysmata vittata* Stimpson, 1860. The basis of their reasoning, however, may have been faulty due to long-standing unresolved species-specific taxonomy and errors in morphological assessments (see Fiedler *et al.*, 2010; d'Udekem d'Acoz, 2000).

Much of the taxonomic uncertainty within *Lysmata* has resulted from conflicting and inconsistent terminology surrounding the status and structure of the accessory branch. It is fairly easy to identify and subsequently count individual articles in species having a long, multi-article accessory branch. In species lacking a multi-article accessory branch, there has been confusion about what constitutes an accessory branch (i.e., uniramous *versus* biramous), and if present, determining if such a branch consists of a clearly defined article (e.g., vestigial, rudimentary, fused; Table 1). Studies have also inconsistently described the shape of the accessory branch (e.g., unguiform, hook-like, stump-like; Table 1).

Lysmata has also been divided into two informal groups based on behavior and coloration patterns: "cleaner shrimps" and "peppermint shrimps." The former group is characterized by striking and contrasting coloration, propensity to form pairs in low density aggregations, and conspicuous "fish-cleaning" behavior (*L. amboinensis* De Man, 1888, *L. debelius* Bruce, 1983, *L. grabhami* Gordon, 1935, and *L. splendida* Burukovsky, 2000). The more species-rich peppermint shrimps are characterized by increased gregariousness and color patterns consisting of varying arrays of red longitudinal, oblique, or transverse markings (e.g., *L. californica* Stimpson, 1866, *L. moorei*, *L. seticaudata* Risso, 1816, *L. vittata*, *L. wurdemanni* Gibbes, 1850). Peppermint shrimps may also engage in cleaning behavior, albeit facultatively (Wicksten, 2009; Vaughan *et al.*, 2018a).

Recent phylogenetic analyses have shown partial support for the historical split between *Lysmata* and *Hippolysmata* vis-à-vis the separation of species with and without a multi-article accessory branch, as well as the split between peppermint and cleaner shrimps (Baeza *et al.*, 2009b; Baeza, 2010; Fiedler *et al.*, 2010; Baeza & Fuentes, 2013). Interpretation of these results regarding the accessory branch should be taken cautiously as the phylogenetic placement of some species has been shown to vary based on sequence alignment strategy, phylogenetic analysis procedure (e.g., *L. bahia* Rhyne & Lin, 2006, *L. californica*, *L. olavoi* Fransen, 1991, *L. nayaritensis* Wicksten, 2000), and the possible misclassification of morphological characters. For example, Fiedler *et al.* (2010: 6–7) recovered a "Short Branch" clade, which included species defined as "ornamented with a short, one-segmented' accessory branch. This definition, as noted above, may be incorrect as it is uncertain if several of the species included in their "Short Branch" clade possess a one-article accessory branch.

Species of *Lysmata* lacking a multi-article accessory branch have generally been placed into one of two distinct clades comprising: 1) the aforementioned "Short Branch" clade of Fiedler *et al.* (2010), which includes the "Tropical-American" clade of Baeza *et al.* (2009b), Baeza (2010), Baeza & Fuentes (2013), Alves *et al.* (2018) and all the cleaner shrimps, and 2) the 'Morpho-variable" clade of Baeza (2010), Baeza & Fuentes (2013), Soledade *et al.* (2013), and Alves *et al.* (2018), which includes species with an unguiform accessory branch (e.g., *L. hochi* Baeza & Anker, 2008), *L. vittata* (the type species for *Hippolysmata*), as well as combinations of the genera *Exhippolysmata* Stebbing, 1915, *Lysmatella* Borradaile, 1915, and *Mimocaris* Nobili, 1903, rendering *Lysmata* paraphyletic (Baeza, 2010; Fiedler *et al.*, 2010; Baeza & Fuentes, 2013; De Grave *et al.*, 2014; Alves *et al.*, 2018). The recent description of *Lysmata arvoredensis* Giraldes, Macedo, Brandão, Baeza & Freire, 2018 (considered a junior synonym of *Lysmata uncicornis* Holthuis & Maurin, 1952 by González-Ortegón *et al.*, (2020)), which possesses an unguiform accessory branch, but is most closely related to the cleaner shrimps (Giraldes *et al.*, 2018; González-Ortegón *et al.*, 2020), has cast further doubt on the evolutionary importance of antennular structure using previously accepted schemes. Multiple studies have nevertheless recovered a well-supported clade of species with a multi-article accessory branch, which includes *L. seticaudata*, the type species for *Lysmata* (Baeza *et al.*, 2009b; Baeza, 2010; Baeza & Fuentes, 2013; Soledade *et al.*, 2013; Alves *et al.*, 2018).

Within *Lysmata*, the species with the longest standing taxonomic ambiguity is likely the peppermint shrimp *L. vittata*, described from material collected in Hong Kong during the North Pacific Exploring Expedition (1853–1856; Stimpson, 1860). *Lysmata vittata* has been reported from across the western Pacific and central Indo-West Pacific, along the Indian Ocean Rim (including South Africa) from the Red Sea to Australia, and in the western Pacific as far north as southeastern Russia (Kemp, 1914; Hale, 1929; Hayashi & Miyake, 1968; Kensley, 1981; Bruce, 1990; Chace, 1997; Marin *et al.*, 2012; De Grave *et al.*, 2014; Anker & De Grave, 2016; Samuel *et al.*, 2016). *Lysmata vittata* is also reported as introduced to Brazil (Soledade *et al.*, 2013), Caribbean Panama (Pachelle *et al.*, 2018) and Mediterranean Egypt (Abdlesalam, 2018), and despite its widespread Indo-West Pacific distribution, as possibly cryptogenic in New Zealand (Ahyong, 2010).

Several species are considered to be synonyms of *L. vittata*, including *Nauticaris unirecedens* Spence Bate, 1888 (type locality: Hong Kong), *Hippolysmata vittata* var. *subtilis* Thallwitz, 1891 (type locality: Philippines), *Hippolysmata durbanensis* Stebbing, 1921 (type locality: South Africa), *Lysmata rauli* Laubenheimer & Rhyne, 2010 (type locality: Brazil), plus an unnamed variety from the Andaman Islands, India (Kemp, 1914). In particular, much uncertainty surrounds the validity of *L. rauli*, which was described from a single individual collected in central Brazil (Laubenheimer & Rhyne, 2010). The authors determined it was a novel species based on a

Table 1. Dorsal antennule and accessory branch structure of *Lysmata* species lacking a well-developed accessory branch. # *Lysmata arvoredensis* is regarded as a junior synonym of *L. uncicornis* by González-Ortegón *et al.* (2020). ^ Status of dorsal antennule not explicitly stated in text, but illustrations indicate a lack of a well-developed accessory branch. * *Lysmata rauli* is regarded as a junior synonym of *L. vittata* by Soledade *et al.* (2013).

Species	Antennule structure	Relevant citations
Lysmata amboinensis (De Man, 1888)	uniramous	Chace, 1997
Lysmata anchisteus Chace, 1972	vestigial	Chace, 1972
	unguiform	Fiedler *et al.*, 2010
Lysmata ankeri Rhyne & Lin, 2006	possessing accessory ramus	Pachelle *et al.*, 2020
Lysmata arvoredensis Giraldes, Macedo, Brandão, Baeza & Freire, 2018	unguiform	Giraldes *et al.*, 2018#
Lysmata bahia Rhyne & Lin, 2006	long accessory ramus	Pachelle *et al.*, 2020
Lysmata baueri Prakash & Baeza, 2017	free distal segment	Prakash & Baeza, 2017
Lysmata boggessi Rhyne & Lin, 2006	[not reported]	Rhyne & Lin, 2006^
Lysmata brevrostrus Wang & Sha, 2018	one segment vestigial	Wang & Sha, 2018
Lysmata californica (Stimpson, 1866)	uniramous	Stimpson, 1866
	fused or with 1 free segment	Wicksten, 2000
Lysmata debelius Bruce, 1983	uniramous	Bruce, 1983
Lysmata dispar Hayashi, 2007	one segmented	Hayashi, 2007
Lysmata grabhami (Gordon, 1935)	absent or vestigial	Gordon, 1935
	single free segment	Pachelle *et al.*, 2020
Lysmata gracilirostris Wicksten, 2000	fused or with 1–2 free segments	Wicksten, 2000
Lysmata guamensis Anker & Cox, 2011	stump like	Anker & Cox, 2011
Lysmata hochi Baeza & Anker, 2008	unguis-shaped	Baeza & Anker, 2008; Jose *et al.*, 2020
Lysmata kempi Chace, 1997	uniramous	Froglia & Deval, 2013
Lysmata kuekenthali (De Man, 1902)	unguis-shaped	Baeza and Anker, 2008
	uniramous	Chace, 1997
	one-segmented finger-liked	Ashrafi *et al.*, 2021
Lysmata leptodactylus Gan & Li, 2016	one segment vestigial	Gan & Li, 2016
Lysmata lipkei Okuno & Fiedler, 2010	unguis-shaped	Okuno & Fiedler, 2010
	one free article	Pachelle *et al.*, 2016
	one-segmented finger-shaped	Ashrafi *et al.*, 2021
Lysmata morelandi (Yaldwyn, 1971)	one-segmented	Yaldwyn, 1971
	vestigial	Hanamura, 2008
Lysmata multiscissa (Nobili, 1904)	one-segmented	Hayashi, 2007
Lysmata nayaritensis Wicksten, 2000	fused or with 1–2 free segments	Wicksten, 2000
Lysmata olavoi Fransen, 1991	vestigial	Fransen, 1991
Lysmata parvispinosus Wang & Sha, 2018	one segment vestigial	Wang & Sha, 2018
Lysmata pederseni Rhyne & Lin, 2006	[Not reported]	Rhyne & Lin, 2006^
Lysmata philippinensis Chace, 1997	one-segmented	Chace, 1997
Lysmata porteri (Rathbun, 1907)	fused	Rathbun, 1907
Lysmata rafa Rhyne & Anker, 2007	rudimentary	Rhyne & Anker, 2007
Lysmata rathbunae Chace, 1970	vestigial	Chace, 1972
Lysmata rauli Laubenheimer & Rhyne, 2010	rudimentary	Laubenheimer & Rhyne, 2010
	unguiform	Soledade *et al.*, 2013*
Lysmata splendida Burukovsky, 2000	uniramous	Burukovsky, 2000
Lysmata stenolepis Crosnier & Forest, 1973	small elongated lobe with 3 articulated bristles	Crosnier & Forest, 1973
Lysmata udoi Baeza, Bolaños, Hernandez & López, 2009	rudimentary	Baeza *et al.*, 2009a
Lysmata uncicornis Holthuis & Maurin, 1952	hook-shaped	Holthuis & Maurin, 1952
	one-segmented	González-Ortegón *et al.*, 2020
Lysmata vittata (Stimpson, 1860)	uniramous	Stimpson, 1860; Bruce, 1990; Marin *et al.*, 2012
	two-segmented	Kubo, 1951
Lysmata wurdemanni (Gibbes, 1850)	rudimentary secondary ramus	Rhyne & Lin, 2006

morphology and coloration pattern distinct from all other western Atlantic *Lysmata*. Soledade *et al.* (2013) nevertheless concluded *L. rauli* was introduced to Brazil, and was in fact a synonym of *L. vittata* due to its morphological similarity to published accounts from the Indo-West Pacific and to genetic similarity to *L. vittata* from Thailand.

Although Soledade *et al.* (2013) presented a detailed morphological analysis of Brazilian *L. vittata* based upon extensive newly-collected material, there was no corresponding analysis of *L. vittata* from across its presumptive native range. The genetic analyses presented in Soledade *et al.* (2013) were limited by a lack of available sequences from the presumably broad range of *L. vittata*.

Their study also highlighted discrepancies in several key morphological characters in the literature for *L. vittata*. Soledade *et al.* (2013) and Alves *et al.* (2018) found that Brazilian *L. vittata* possessed an unguiform accessory branch. Stimpson's (1860) original description, the redescription by Bruce (1990) of the species based on a single ovigerous individual collected in Hong Kong (type locality), and material from the Sea of Japan (Marin *et al.*, 2012) nevertheless all describe the dorsal antennule as uniramous.

Coloration patterns have been used as an important character to delimit *Lysmata* species (Rhyne & Lin, 2006; Rhyne *et al.*, 2012; Baeza & Behringer, 2017; Pachelle *et al.*, 2020), and patterns described for *L. vittata* appear to vary geographically. Photographs of *L. vittata*/*L. rauli* from Brazil in Laubenheimer & Rhyne (2010), Soledade *et al.* (2013), Almeida *et al.* (2018), and Alves *et al.* (2018, 2019) show individuals with numerous fine red stripes, with dark transverse bands at the anterior end of the first somite and between the third and fourth somites. This agrees with other reports of *L. vittata* from Australia (Vaughan *et al.*, 2018b; Barton *et al.*, 2020), India (Kemp, 1914), Caribbean Panama (Pachelle *et al.*, 2018), Singapore (Anker & De Grave, 2016), and Mediterranean Egypt (Abdlesalam, 2018). In contrast, photographs and illustrations of *L. vittata* from Japan (Hayashi & Miyake, 1968) and southeastern Russia (Marin *et al.*, 2012) document individuals with numerous red stripes, but without any transverse bands.

There has also been different accounts of the number of carpal articles in the second pereopod in *L. vittata*. Chace (1997) reported 15–31 articles, notable as being far greater than typically reported in lysmatine shrimps. Anecdotal data suggest *L. vittata* that possess transverse bands as described above and/or unguiform accessory branch generally have lower carpal article counts of the second pereopod, 15–18 (Alves *et al.*, 2018), 15–19 (mean 16; Soledade *et al.*, 2013), 15–19 (Kemp, 1914; Pachelle *et al.*, 2020), *versus* those lacking transverse bands and/or unguiform accessory branch, 20 (Bruce, 1990), 19–22 (Hayashi & Miyake, 1968), and 18–21 (Ahyong, 2010). Limited data also suggest there may be differences in zoeal morphology in *L. vittata* collected from southeastern Russia and Korea in comparison with India (Yang & Kim, 2010; Marin *et al.*, 2012).

Given the presumptive wide geographic range and notable differences in morphological descriptions (particularly in relation to the presence/absence of an accessory branch, number of carpal articles in the second pereopod, and coloration patterns), *L. vittata* likely represents a species complex (Chace, 1997; Marin *et al.*, 2012; Anker & De Grave, 2016; Pachelle *et al.*, 2018). Further confounding this taxonomic uncertainty is the brief original description of Stimpson (1860), which lacked illustrations, and the destruction of type material during the Great Chicago Fire of 1871 (Dawn Roberts, Chicago Academy of Sciences, personal communication, 2016), as well as the destruction of type material of *H. vittata* var. *subtilis* during the aerial bombing of Dresden in World War II (André Reimann, Senckenberg Natural History Collections of Dresden, personal communication, 2017). The type material for *N. unirecedens* is in poor condition, including missing all antennules (as also noted by De Man (1907)) as well as a majority of pereopods (RA, personal observation).

Taxonomic uncertainly also surrounds the status of *L. dispar* Hayashi, 2007 and *L. lipkei* Okuno & Fiedler, 2010. *Lysmata dispar* was described from the Dampier Archipelago, Australia from preserved material and we are unaware of any further records for this species. Okuno & Fiedler (2010) described *L. lipkei* from central and southern Japan, which included photographs of the holotype in fresh condition. The morphological differences between these two species are subtle and include length and curvature of the rostrum, presence/absence of a minute spinule on the distal article of the antennular peduncle, and articulation of the ischium of the second pereopod. At about the same time of the arrival of *L. vittata* in Brazil, another Western Pacific *Lysmata* was found in that country. This second *Lysmata* displayed characters of both *L. dispar* and *L. lipkei*. Pachelle *et al.* (2016) and Alves *et al.* (2018) settled on *L. lipkei* as the name for this Brazilian introduction based on the examination of paratypes of *L. lipkei* and genetic and color pattern similarity to *L. lipkei* from Japan. Due to the ambiguity of the proposed diagnostic characters, *L. lipkei* could represent a junior synonym of *L. dispar*.

Unidentified, reproductively active peppermint shrimp have been collected at multiple locations in the lower Chesapeake Bay, eastern USA since 2013, as well as in adjacent coastal embayments near Wachapreague, Virginia. These shrimp were morphologically distinct from the sole native *Lysmata* species in the region, *L. wurdemanni* Gibbes, 1850 and from other related Atlantic species. Instead, all specimens examined were in agreement with Stimpson's (1860) original description and the redescription of *L. vittata* from Hong Kong by Bruce (1990). To help resolve taxonomic ambiguity, we designate, as a neotype, the specimen used in the redescription of *L. vittata* by Bruce (1990). With the neotype fixed, we document the first report of *L. vittata* sensu stricto in North American waters and provide the first phylogeny incorporating individuals from across the introduced and presumably native ranges of *L. vittata*. We also provide brief morphological comparisons among specimens from across a large portion of the species current range. These data clearly confirm that *L. vittata* sensu lato is a species complex. We also identify the putative native range for shrimp collected in the Chesapeake region, which will aid in assessing invasion potential at larger scales. Further studies are required to correctly understand previously described ostensible junior synonyms in order to fully delimit this species complex.

We used the following abbreviations in the text: FLMNH, Florida Museum of Natural History, Gainesville, FL, USA; MAGNT, Museum and Art Gallery Northern Territory, Darwin, NT, Australia; NHM, The Natural History Museum, London, UK; MZUSP, Museum of Zoology of the University of São Paulo, São Paulo, Brazil; NIWA, National Institute of Water and Atmospheric Research, Wellington, New Zealand; NMNH, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; NTM, museum collection prefix for MAGNT; SERC, Smithsonian Environmental Research Center, Edgewater, MD, USA; UF, museum collection prefix for FLMNH; USNM, museum collection prefix for NMNH; VIMS, Virginia Institute of Marine Science, Gloucester Point, VA, USA.

MATERIALS AND METHODS

Sample collection and morphological examination

Individuals of *Lysmata* used in this study include both recently collected "fresh" specimens and specimens obtained from museum collections. Fresh North American material was collected as part of the Chesapeake Bay Barcode Initiative (CBBI), one of the largest efforts to create regional verified and vouchered DNA barcode libraries for fish and macroinvertebrates (Aguilar *et al.*, 2017). These specimens were captured by bottom trawl, benthic sled or ARMS (autonomous reef monitoring structures) during CBBI sampling or were provided by VIMS Trawl Survey (Tuckey & Fabrizo, 2013), Blue Crab Winter Dredge Survey, and Smithsonian Institution-led biodiversity surveys (Leray & Knowlton, 2015). We also obtained one individual from Hong Kong collected during on-going biodiversity surveys and six individuals from northern New Zealand collected during port surveys, five of which were previously reported in Ahyong (2010). After collection, specimens were either frozen or placed in 95% non-denatured ethanol and capture metadata (date, GPS coordinates, gear, salinity, water temperature, and depth) were recorded. We obtained museum specimens of *Lysmata vittata* from MAGNT collected from 1980–1992. All MAGNT specimens were from northern Australia, except for the topotypic material from Hong Kong (Bruce, 1990).

For all specimens of *Lysmata*, the following morphological characters were studied: 1) position of the rostrum tip in relation to the antennular peduncle, 2) number of dorsal and ventral rostral teeth, 3) presence or absence of the pterygostomial spine, 4) length of antennal scale (scaphocerite) in relation to antennular peduncle, 5) length/width ratio of antennal scale, 6) stylocerite length, 7) number of carpal articles on the second pereopod, and 8) status of the accessory branch. We also noted when possible the coloration patterns of all freshly collected, unpreserved specimens.

DNA sample preparation

Fresh North American samples were processed using standard CBBI protocols (Aguilar *et al.*, 2017) as follows. If frozen, samples were first thawed. All specimens were photographed and two tissue samples (typically pleopods or sections of a pereopod, but also eggs of ovigerous individuals) were excised. To avoid contamination, all tools and work surfaces were sanitized prior to tissue processing by exposure to a 10% bleach solution for at least 10 min, followed by a thorough rinsing with reverse-osmosis (RO) treated water and drying. Each shrimp was handled with sanitized forceps and scalpel, which only handled a single specimen and were sanitized before any additional uses; specimens were processed on an unused area of the sanitized workspace. After all the unused portions of the work surface were used or all samples on-hand were processed, the work surface was sanitized before any additional uses. One tissue sample was placed in a sterile 0.75 ml microcentrifuge tube containing 150 µl of TD-M2 extraction buffer (Autogen, Holliston, MA, USA) and frozen until DNA amplification and sequencing. The other tissue sample was placed in a sterile 1 ml tube containing a dimethyl sulfoxide (DMSO) solution or 95% non-denatured ethanol to serve as a tissue voucher.

Physical voucher specimens of the North American material were placed in 95% ethanol and deposited in the invertebrate zoology collections of NMNH or FLMNH (Supplementary material Table S1). All photographic vouchers, metadata, and subsequent DNA sequences and trace files were uploaded to the Barcode of Life Database (BOLD; Ratnasingham & Hebert, 2007). All North American DNAs and tissue vouchers were transferred to the Smithsonian Tissue Biorepository for storage and are available upon request. The Hong Kong and New Zealand vouchers were transferred to the collections of the FLMNH and NIWA, respectively (Supplementary material Table S1). DNA sequences and corresponding metadata were also uploaded to GenBank (OK662574-OK662576, OL664568-OL664577, OL690297-OL690313, Supplementary material Table S1). Corresponding COI sequences for North American, New Zealand, and Australian museum material, plus additional North American *L. vittata* not included in this study were also uploaded to GenBank (KP254053, KP254224, KP254337, KP254474, KP254481, KP254584, KU905757, MH143252-MH143270, MZ836204-MZ836205, OL664562, OL664564-OL664567). Museum specimens were processed identically as fresh North American material, except that no photographic or tissue vouchers were taken.

DNA amplification and sequencing

DNA was extracted using the Autogen Prep 965 phenochloroform automated extractor (Autogen, Holliston, MA, USA). For fresh North American samples, we amplified a ~500 bp region of the mitochrondrial 16S rRNA gene using the universal primers 16Sar (CGCCTGTTTATCAAAAACAT) and 16Sbr (CCGGTCTGAACTCAGATCACGT) of Palumbi *et al.* (1991). The 16Sar/16Sbr primer sets performed poorly with the 20–30 year-old ethanol preserved museum material. To increase sequencing success, we designed *Lysmata*-specific primers, 16SF_Lysmata (GCGGTAYHHTGACCGTGCRAAGG) and 16SR_Lysmata (TTAATTCAACATCGAGGTCGC), in Geneious Prime v.2020.1.1 (Biomatters, San Francisco, CA, USA) targeting a 360 bp fragment of the 16S rRNA gene to increase the amplification efficiency from the more degraded DNA of these historical samples. Amplification reaction cocktails for 16Sar/16Sbr contained 1 µl 10X reaction guffer (Bioline, Cincinnati, OH, USA), 0.3 µM each primer, 2.5 mM MgCl, 0.5 mM dNTP, and 0.5 units Biolase Taq (Bioline), in a 10 µl final volume. Cocktails for the *Lysmata*-specific primers contained 5 µl 2× GoTaq HotStart Master Mix (Promega, Madison, WI, USA), 20 µg BSA, and 0.3 µM of each primer, in a 10 µl final volume. The thermocycler profile for the 16Sar/16Sbr reactions started with a denaturation at 95 °C for 7 min, followed by 35 cycles of 95 °C 30s, 50 °C 30s, 72 °C 45s, finishing with an extension of 72 °C for 90s. The profile for the *Lysmata*-specific primers was identical except for an annealing temperature of 54 °C. PCR products were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) following manufacturer's instructions. Sequencing was performed using the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) following manufacturer's instructions. Sequencing was carried out with a 3730xl DNA Analyzer (Applied Biosystems) at the Laboratories of Analytical Biology (LAB), NMNH.

Chromatograms were processed using Sequencher 5.0.1 (Gene Code Corporation, Ann Arbor, MI, USA). Each was subjected to the following, very conservative, trimming parameters: trim until the first and last 25 bp contain fewer than three ambiguities and trim until the first and last 10 bp contain fewer than three bases with a Phred score below 30. Only trimmed fragments greater than 200 bp in length and with overall confidence above 90% (as calculated by Sequencher) were used to construct the final sequences. Passing sequences were visually examined for errors.

The fresh Hong Kong material was processed like the North American material, with the exception that the DNA was extracted by hand using a standard phenol-chloroform extraction protocol and was amplified using PCR Master Mix (Thermo Scientific, Waltham, MA, USA) with an added 32 µg BSA, and 0.3 µM of each *Lysmata*-specific primer in a final volume of 25 µl. The Hong Kong sample was then sequenced at BGI (Beijing Genomics Institute, Schenzen, China). The New Zealand material was processed similarly to the fresh North American material, except that it was sequenced at Macrogen (Seoul, South Korea).

Phylogenetic analyses and species delimitation

In addition to the 30 16S rRNA gene sequences generated, we downloaded 39 16S rRNA gene sequences from GenBank, including 32 sequences from 24 different *Lysmata* species, four sequences of additional lysmatids (*Exhippolysmata ensirostris* Kemp 1914, *Ligur ensiferus* Risso, 1816, *Lysmatella prima* Borradaile, 1915, and *Mimocaris heterocarpoides* Nobili, 1903), and three sequences of non-lysmatid shrimps (*Merguia oligodon* De Man, 1888, *Merguia rhizophorae* Rathbun, 1900, and *Tozeuma carolinense* Kingsley, 1878; Supplementary material Table S1). To improve resolution, we preferentially selected GenBank sequences with detailed collection metadata and associated voucher specimens whenever possible.

Sequences were aligned in Geneious Prime v.2020.1.1 using the MAFFT (Katoh *et al.*, 2002; Katoh & Toh, 2008) plugin with the default parameters. The resulting alignment contained 69 sequences and was 776 base pairs (bp) in length. The alignment was exported with the missing ends of the alignment filled with N, to indicate missing data, then run in the Gblocks online server (Castresana, 2000) with the following relaxed parameters allowing: 1) smaller final blocks, 2) gap positions within the final blocks, and 3) less strict flanking positions. We used the IQtree online server (Trifinopoulos *et al.*, 2016) to determine the appropriate substitution model for the resulting alignment from Gblocks, which contained 490 bp, using the Akaike information criteria corrected values (AICc) and Bayesian

information criteria (BIC) methods. Both resulted in the same substitution model, TVM+F+I+G4, which was used to generate a parsimony tree, maximum likelihood (ML), in the IQtree online server using the ultrafast bootstrap method with 1,000 bootstrap replicates and a minimum correlation coefficient of 0.99. We used the same alignment to generate a second phylogenetic tree using the MrBayes (Ronquist & Huelsenbeck, 2003) plugin in Geneious Prime with the closest available substitution model, GTR + I with four gamma categories and default parameters. For the Bayesian Inference (BI) analysis, unique random starting trees were used in the Metropolis-coupled Markov chain Monte Carlo (MCMC; see Huelsenbeck & Ronquist, 2001). The analysis was performed with random seeding for 1.1 million generations. A burn-in of 100,000 trees was conducted, then every 200th tree was sampled. A consensus tree was calculated for the 4,951 sampled trees. Uncorrected *p*-distances were estimated using Mega v7.0 (Kumar *et al.*, 2016).

To further examine relationships between *L. vittata*/*L. rauli* and *L. dispar*/*L. lipkei* we used automatic barcode gap discovery (ABGD; Puillandre *et al.*, 2012), a species-delimitation strategy that quantitatively identifies the optimal intraspecific threshold or "barcoding gap" in a given dataset, which can be used to partition individual samples into candidate species (Martínez-Arce *et al.*, 2020). The ABGD analysis was conducted via the online ABGD server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) using the same alignment employed in our phylogenetic analyses and the Kimura (K80; TS/TV = 2) substitution model (Kimura, 1980). We used all default parameters (Pmax = 0.1; Steps = 10; Relative Gap Width [X] = 1.5; Number of bins = 20), except for a Pmin of 0.01.

RESULTS

Field collection and morphological study of North American material

From 4 May 2013 to 28 July 2018, we collected 165 lysmatid shrimp from the lower Chesapeake Bay and adjoining embayments (Fig. 1; Supplementary material Table S2) that were morphologically identified as *L. vittata* according to Stimpson (1860), Bruce (1990), and Chace (1997). A subset of 80 individuals were examined in fine detail. These specimens displayed the following characters: 1) uniramous dorsal antennule, 2) rostrum reaching the distal end of second antennular article, 3) antennal scale reaching the end of the antennular peduncle, 4) stylocerite reaching the mid-length of the proximal antennular article, 5) pterygostomial spine, and 6) carpus of the second pereopod with 18–23 articles (Figs. 2, 3; Table 2). The body was semi-transparent with thin reddish stripes. The abdomen possessed thin red longitudinal stripes, which alternated in pigment density, without any oblique or transverse bars (Fig. 2). Ovigerous individuals possessed yellow or green eggs.

Lysmata vittata is easily distinguished from *L. wurdemanni*, the only native species of *Lysmata* in the Chesapeake Bay region, by the presence of a pterygostomial spine, lower number of carpal articles of the second pereopod (< 30), relatively shorter antennal scale that does not distinctly overreach the antennular peduncle (3:1 length-width ratio), and a coloration pattern that does not include oblique bars on the abdomen (Table 2). Although our North American specimens of *L. vittata* bore some superficial resemblance to the original description of *L. rauli* (Laubenheimer & Rhyne, 2010) and subsequent analysis of additional Brazilian material of *L. rauli* re-classified as *L. vittata* (Soledade *et al.*, 2013; Alves *et al.*, 2018), as well as *L. vittata* from Egypt (Abdlesalam, 2018), there were clear and consistent morphological differences between the North American *L. vittata* and *L. rauli*/*L. vittata* from South America and the Mediterranean. Most prominent was the absence of a biramous dorsal antennular flagellum in the former. The North American *L. vittata* also possessed a relatively longer rostrum, reaching the distal end of the second article of the antennular peduncle, as opposed to reaching the midpoint of the same article, and tended to have higher second pereopod carpal article counts (18–23 *versus* 15–19). All North American *L. vittata* also lacked red transverse bars on the abdomen, unlike *L. rauli*/*vittata* from Brazil (Laubenheimer & Rhyne, 2010; Soledade *et al.*, 2013; Alves *et al.*, 2018, 2019), Mediterranean (Abdlesalam, 2018), India (Kemp, 1914), Caribbean Panama (Pachelle *et al.*, 2018), and Singapore (Anker & De Grave, 2016). The North American *L. vittata* were also morphologically similar to the specialized cleaner shrimp *L. amboinensis* and *L. grabhami*, but they possess a shorter stylocerite (reaching one-fourth to one-third of the length of the proximal article) and relatively longer antennal scale (3.5–5.0 times length to width). In life, these species are clearly distinguished by unmistakable differences in coloration.

The North American *L. vittata* were collected throughout a large portion of the lower Chesapeake Bay, including the lower reaches of the James and York rivers and from Burtons Bay, a coastal embayment near Wachapreague, Virginia (Fig. 1; Supplementary material Table S2). Habitats were variable, ranging from shallow sand flats/oyster reef (0.5 m) to deeper muddy areas near the main channels (19.5 m). Salinities ranged from 18.48 psu to near full strength seawater (34 psu) in Burtons Bay. During the study period, *L. vittata* were captured across all seasons with bottom water temperatures ranging from 4.49–26.5 °C. In a large number of samples the congener *L. wurdemanni* were also collected concurrently with *L. vittata*.

Morphological analysis of museum and additional material

Preliminary analysis of material of *L. vittata* obtained from MAGNT indicated that the collections comprised several distinct species with widely different morphologies. We then selected a subset of 10 individuals from seven separate lots for detailed morphological and genetic analyses (original and new museum numbers are listed in Supplementary material Table S1). One of the selected shrimp was the same individual used in the redescription of *L. vittata* (NTM Cr004983) by Bruce (1990). Our examination completely agreed with the analysis of Bruce (1990) and with our North American *L. vittata* (including the possession of a uniramous dorsal antennule (Fig. 3)), as well as with Stimpson (1860) and Chace (1997); it was therefore identified as *L. vittata*.

The remaining individuals were dissimilar and appeared to represent three separate species. Five individuals from four separate lots (NTM: Cr004808, Cr004904, Cr009751, and Cr009787) were most similar to the original description of *L. rauli* and *L. vittata* from Brazil and Egypt, with some minor differences, and were identified as *L.* cf. *rauli*. These shrimp all possessed a one-article accessory branch (Fig. 3), rostrum that reached the distal end of the second article of the antennular peduncle, antennal scale that reached the end of the antennular peduncle (3 times long as wide), stylocerite that reached the mid-length of proximal article of antennular peduncle, pterygostomial spine, and 17–26 carpal articles of the second pereopod. Individuals nevertheless differed from Brazilian *L. vittata* by having a longer rostrum, which reached the distal end, rather than the mid-length, of the second antennular article and had generally higher second pereopod carpal article counts (Table 2).

Three individuals from a single lot (NTM Cr009616) were identified as *L. dispar*. The morphological differences between *L. dispar* and *L. lipkei* are minor and predominantly subjective. All three individuals shared traits attributed to both species. They possessed a minute spinule on the distal antennular peduncle article as with *L. lipkei*. Conversely, the articulations of the ischium of the second pereopod were distinct, as with *L. dispar*. Okuno & Fiedler (2010) described the ischial articles of the second pereopod as barely visible in *L. lipkei*, whereas the same articles are distinct in *L. dispar* (Hayashi, 2007). The ischial articles were clearly noticeable in the present material, albeit less distinct than the meral and carpal articles. The rostrum was straight (similar

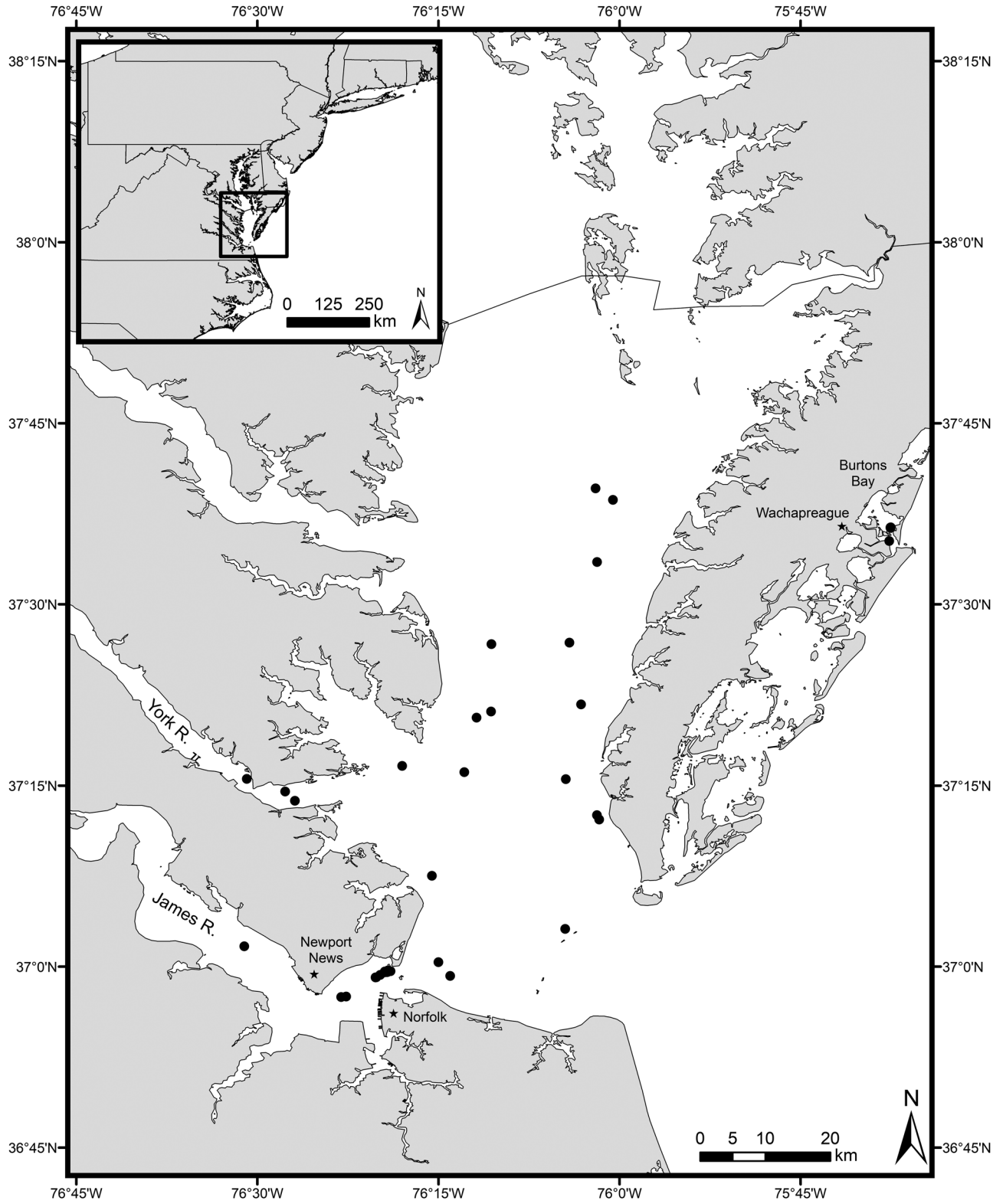

Figure 1. The greater Chesapeake Bay region indicating capture locations (black dots) of *Lysmata vittata*, 2013–2018. Inset represents the location of the lower Chesapeake Bay in relation to the mid-Atlantic region of the United States.

to *L. lipkei*) in two of the three specimens and slightly convex (similar to *L. dispar*) in the third. The shape of the rostrum is a highly variable character and should be taken with caution, particularly when the reported differences between species are extremely slight.

Given that our material shared characters associated with *L. dispar* and *L. lipkei* and the general taxonomic uncertainty surrounding both species, it is difficult to assign a species designation with absolute certainty. Our material was collected in the vicinity of the type locality of *L. dispar* (northern Australia)

and in the event of future synonymy with *L. lipkei*, the earlier described *L. dispar* would have precedence; thus, we felt *L. dispar* was a better determination. The Australian *L. dispar* also possessed a rostrum with 6 dorsal and 2 to 3 ventral teeth, which reached or barely reached the distal end of the second article; one-article accessory branch; long stylocerite, nearly reaching the distal end of the first antennular article; chelae of first pereopod with concave cutting edges proximally, which make a narrow gap when closed; 29–30 second pereopod carpal articles; and a pterygostomial spine.

The last individual was identified as an unknown species of *Lysmata*, which possessed a uniramous dorsal antennule, an antennal scale that reached just beyond antennular peduncle, a short stylocerite not reaching midpoint of first antennular article (a fairly unique character among *Lysmata*), noticeably slender second pereopod with 33 carpal articles (one second pereopod was missing), and a pterygostomial spine. The rostrum was broken, but four dorsal teeth were present on the body midline. This combination of traits does not match any described *Lysmata* species. It was similar to *L. gracilirostris* Wicksten, 2000, which also possesses a short stylocerite, as well as a relatively high second pereopod carpal-article count (28–31), pterygostomial spine, and lacks a multi-article accessory branch, but the antennal scale is longer, reaching well past the antennular peduncle. Similarly, both *L. californica* and *L. olavoi* possess a much longer antennal scale (reaching well past the antennular peduncle), as well as a longer stylocerite (reaching to or past the midpoint of the first antennular article). Of the six *Lysmata* species reported from Australia by Hayashi (2007) and Hanamura (2008) (*L. amboinensis*, *L. dispar*, *L. morelandi* Yaldwyn, 1971, *L. multiscissa* Nobili, 1904, *L. ternatensis*, and *L. vittata*), it was most similar to *L. multiscissa* sensu Hayashi (2007), with the important exception that Hayashi (2007) noted the presence of a one-article accessory branch, absent in this specimen.

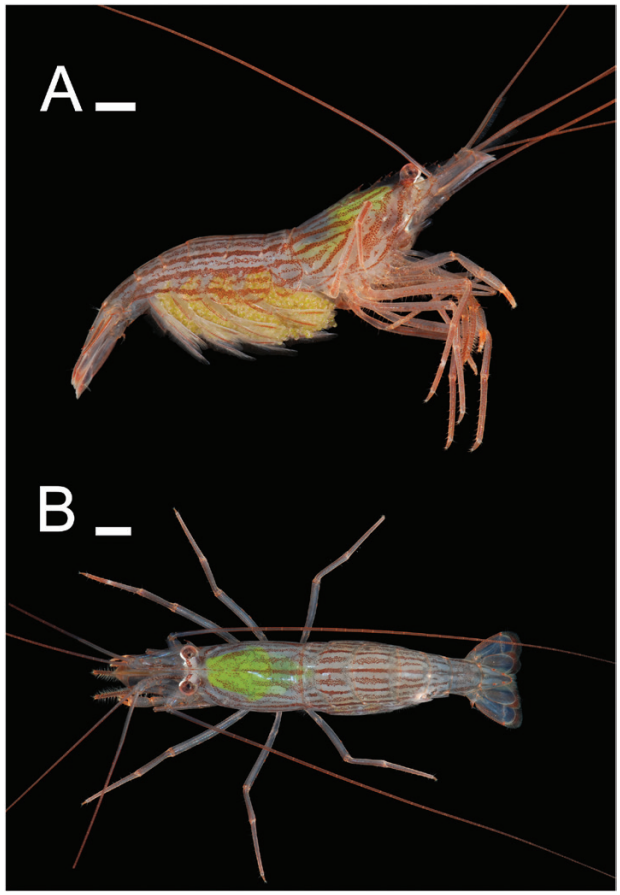

Figure 2. Lateral (**A**) and dorsal (**B**) views of an ovigerous specimen of *Lysmata vittata* collected on 25 July 2018 in Wachapreague Channel, VA (UF 58904). Scale bar = 3 mm. Photo by RA.

We re-examined five of the seven specimens of *L. vittata* (all but NIWA [MITS] 13209) collected in northern New Zealand (Auckland and Kaipara Harbor) previously reported in Ahyong (2010), plus an additional *L. vittata* collected from Whangārei, New Zealand (NIMA [MITS] 41459). Our analyses confirm the original identifications and all individuals completely agreed with our North American material, as well as with Stimpson (1860), Bruce (1990), and Chace (1997) (Table 2). We also examined one individual collected in Hong Kong (UF 047210), which was tentatively identified as *L rauli*. It was most similar to the original description of *L. rauli*, as well as *L. vittata* from Brazil and Egypt. This specimen possessed a one-article accessory branch, rostrum that reached the midpoint of the second article of the antennular peduncle, antennal scale that reached the end of the antennular peduncle (three times long as wide), stylocerite that reached the mid-length of proximal article of the antennular peduncle, pterygostomial spine, and < 25 carpal articles of the second pereopod. The voucher unfortunately went missing before a full quantitative analysis could be performed. This suite of morphological characters nevertheless was confirmed in a subsequent examination of the *L. rauli* holotype (USNM:IZ:1130395). The live coloration of this shrimp from Hong Kong included numerous fine red stripes with transverse red bands at the first somite and between the third and fourth somites, similar to photos of the *L. rauli* holotype in Laubenheimer & Rhyne (2010).

Phylogenetic analyses and species delimitation

Within our initial 776 bp alignment, all individuals of *L. rauli*/*L. vittata* from Brazil, Hong Kong, and Thailand had two insertions (18 and 11bp) in the middle of the 16S sequences. Similar insertions were previously noted in sequences of Brazilian *L. vittata* and *L. bahia* (Baeza, 2010). These insertions were removed by Gblocks and were not included in the final alignment used to

Figure 3. Photographs of the dorsal antennule of selected species of *Lysmata* shrimps. *Lysmata* cf. *rauli* (Indian Ocean, Western Australia; NTM Cr019373) (**A**); *Lysmata vittata* (neotype, Hong Kong; NTM Cr004983) (**B**); *Lysmata vittata* sensu stricto (Wachapreague, VA, USA; USNM:IZ:1463186 (**C**). Images captured with a Hirox HRX-01 Digital Microscope (Hirox USA, Hackensack, NJ, USA). Photos by RA.

Table 2. Variation in relevant morphological characters of members of the *Lysmata vittata* species complex and *Lysmata wurdemanni*.*Pachelle *et al* (2020) reported a single individual of *L. vittata* from Brazil (MZUSP 31541) with a carpal second pereopod count of 22–23 (and a meral count of 12–13), considerably higher than has previously been reported.

Character	*Lysmata vittata* – Hong Kong	*Lysmata vittata*– USA	*Lysmata vittata* – New Zealand	*Lysmata vittata* – Brazil	*Lysmata cf. rauli*– Australia	*Lysmata wurdemanni*
	(Stimpson, 1860; Bruce, 1990; Chace, 1997)	(present study; *N* = 80)	(present study; *N* = 6; Ahyong, 2010)	(Laubenheimer & Rhyne, 2010; Soledade *et al.*, 2013; Pachelle *et al.*, 2020)	(present study; *N* = 6)	(Rhyne *et al.*, 2006)
Number of dorsal rostral teeth	6	6–8	7	6–8	8	4–6
Number of ventral rostral teeth	3	2–6	2–4	2–5	2–4	2–6
Rostrum length	Reaching distal margin of 2nd article of the antennular peduncle	Reaching distal margin of 2nd article of the antennular peduncle	Reaching distal margin of 2nd article of the antennular peduncle	Reaching midpoint of 2nd article of antennular peduncle	Reaching distal margin of 2nd article of the antennular peduncle	Reaching distal margin of 2nd article of the antennular peduncle
Pterygostomial tooth	Present	Present	Present	Present	Present	Absent
Number of articles on the accessory branch of the dorsal antennular flagellum	0	0	0	1	1	0
Stylocerite length	Short, reaching mid length of 1st article	Short, reaching mid length of 1st article	Short, reaching mid length of 1st article	Short, reaching mid length of 1st article	Short, reaching mid length of 1st article	Short, not reaching distal margin of 1st article
Antennal scale (scaphocerite)	Overreaching antennular peduncle, 3× long as wide	Overreaching antennular peduncle, 3× long as wide	Overreaching antennular peduncle, 3x long as wide	Overreaching antennular peduncle, 3× long as wide	Overreaching antennular peduncle, 3× long as wide	Distinctly overreaching antennular peduncle, 4× long as wide
Number of second pereopod carpal articles	15–31	18–23	19–21	15–19 (22–23)*	17–26	30–33
Coloration	Numerous fine red stripes	Thin red stripes alternating in pigment density, without transverse bands	Undetermined	Thin red stripes, with transverse red bands at 1st and between 3rd and 4th somites	Undetermined	Thin red longitudinal stripes with oblique banding ventrally

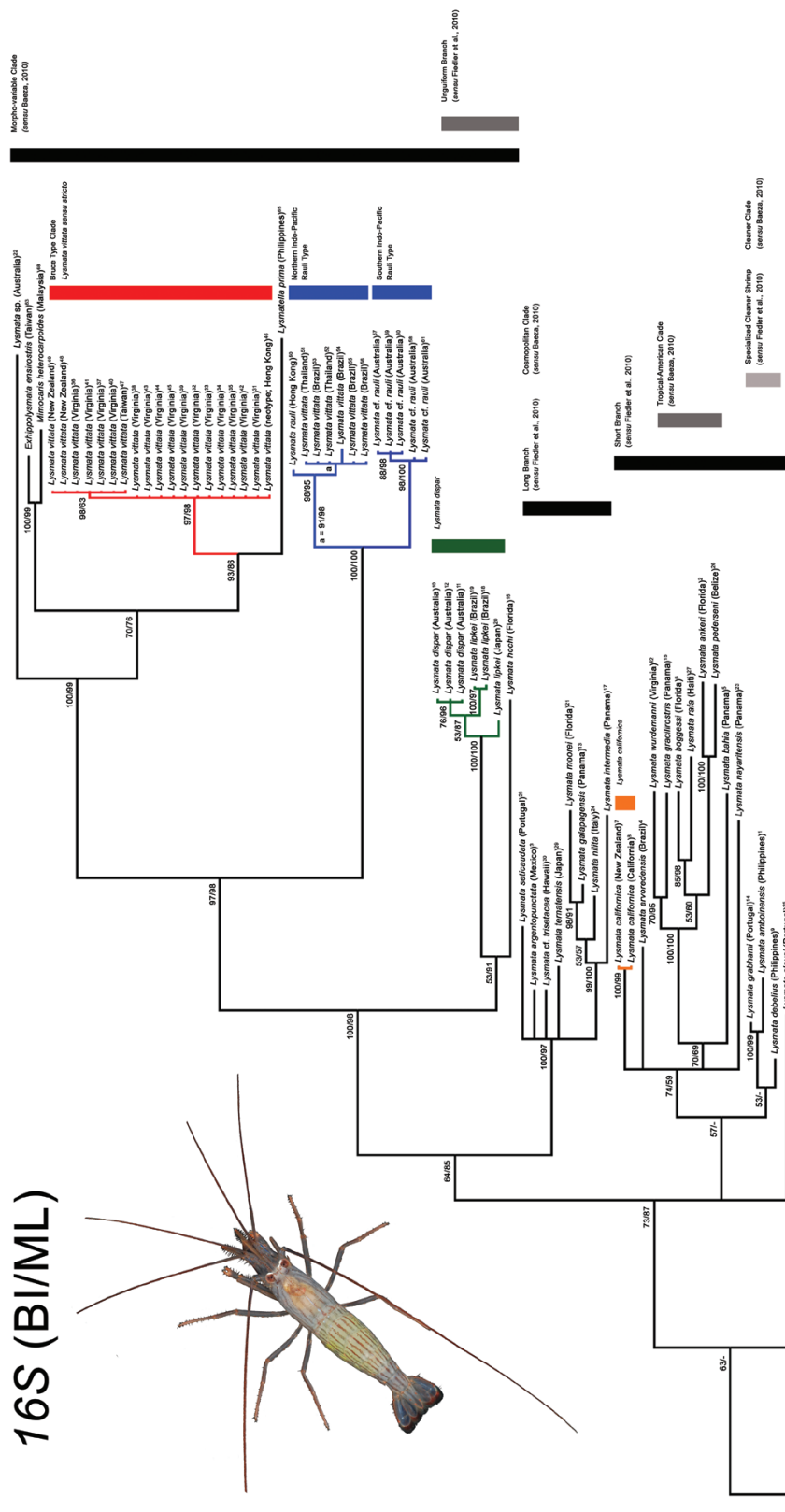

Figure 4. Phylogenetic tree obtained by Bayesian inference (BI) analysis of the partial 16S rRNA gene for shrimps in the genus *Lysmata* and other selected taxa from the families Hippolytidae and Merguiidae. Numbers near branch nodes represent posterior probabilities from the BI analysis and bootstrap values obtained from maximum likelihood (ML) analysis, respectively. Bootstrap values < 50% are not shown.

generate the phylogenetic trees. Both phylogenetic trees (Baysian and ML) produced similar topologies. The uncertain basal position of *L. ensiferus* suggests that Lysmatidae Dana, 1852 may be paraphyletic. The well-supported clade containing both *Merguia* species (posterior probability; p*P* = 100; ML = 100) was sister to the clade comprising all *Exhippolysmata*, *Lysmatella*, *Lysmata*, and *Mimocaris*, though with moderate to low support (p*P*= 63; ML = < 50). Overall, our phylogenetic analyses indicate that within this broader *Lysmata* clade there were three main subclades, all having been previously recognized.

All phylogenetic analyses failed to recover *L. vittata*, including the junior synonym *L. rauli*, as a monophyletic group (Fig. 4). Both the BI and ML analyses of the 16S rRNA gene recovered three strongly supported clades of *L. vittata* sensu lato. The first strongly supported clade, "Bruce Type," comprised all individuals of *L. vittata* from the greater Chesapeake Bay region, New Zealand, Taiwan, as well as the individual from Hong Kong used by Bruce (1990) to redescribe *L. vittata* (p*P* = 97; ML = 98), which was sister to *L. prima* (p*P* = 93; ML = 86). All specimens of the "Bruce Type" clade were examined by the authors (except for the individual from Taiwan) and possessed a uniramous dorsal antennule. The sequence divergence between the "Bruce Type" clade and the other clades of *L. vittata* sensu lato were considerable with *p*-distances ranging from 0.186–0.204 (Supplementary material Table S3). The remaining groups, broadly called "Rauli Type", comprised two sister clades (p*P* = 100; ML = 100), which contained individuals possessing a one-article accessory branch and were morphologically similar to the description of *L. rauli*. The first subclade, further defined as "Northern Indo-West Pacific Rauli Type" comprised individuals from Thailand, Hong Kong, and Brazil. The second subclade, further defined as "Southern Indo-West Pacific Rauli Type" comprised individuals from northern Australia. The genetic differences (*p* distances) between the two "Rauli Type" clades (0.093–1.04) were greater or similar to a number other *Lysmata* species comparisons, including *L. amboinensis*/*L. grabhami* (0.043), *L. debelius*/*L. grabhami* (0.54), *L. galapagensis*/*L. nilita* (0.068), *L. argentopunctata*/*L. ternat ensis* (0.082), *L. galapagensis*/*L. moorei* (0.86), *L. ankeri*/*L. pederseni* (0.09), *L. boggessi*/*L. rafa* (0.093), *L. intermedia*/*L. nilita* (0.104), and *L. gracilirostris*/*L. wurdemanni* (0.0108), as well as between *M. oligodon* and *M. rhizophorae* (0.043; Supplementary material Table S3). The ABGD analysis supported our phylogenetic analyses and delimited the same three strongly supported clades of *L. vittata* sensu lato at all partition levels (i.e., "Bruce Type" and both "Rauli Type" clades; Fig. 4).

All individuals of *L. dispar* and *L. lipkei* were recovered in a strongly supported clade (p*P* = 100; ML = 100). Genetic distances within this clade ranged 0.022–0.025 with the Australian *L. dispar* most similar to *L. lipkei* from Brazil (*p* distance = 0.022; Supplementary material Table S3). The recursive partition delimited three groups within this clade corresponding with geographic origin (i.e., *L. dispar* from Australia, *L. lipkei* from Brazil, and *L. lipkei* from Japan), albeit only at the first partition (proximal maximum distance 0.01).

The genetic sequence of the *L. californica* collected in New Zealand was highly similar to the *L. californica* collected from the species' native range (California; *p*-distance = 0). Both sequences formed a well-supported clade (p*P* = 100; ML = 99), which was delimited as a single putative species in the ABGD analysis at all partition levels (Fig. 4).

All the species of *Lysmata* described as possessing an unguiform accessory branch, except for *L. arvoredensis* (*L. hochi*, *L. lipkei*, and *L. rauli* sensu lato), as well as *L. dispar*, *L. vittata*, the unknown *Lysmata* species, and the non-*Lysmata* species, *E. ensirostris*, *L. prima*, and *M. heterocarpoides*, were recovered in a strongly supported clade (p*P* = 100; ML = 98), rendering *Lysmata* paraphyletic. This first monophyletic clade was analogous to the "Morpho-variable" clade of Baeza (2010; Fig. 4). The second highly supported monophyletic clade (p*P* = 100; ML = 97) consisted of all species of *Lysmata* possessing a multi-article accessory branch (*L. argentopunctata*, *L. galapagensis*, *L. intermedia*, *L. moorei*, *L. nilita*, *L. seticaudata*, *L. ternatensis*, and *L.* cf. *trisetacea*) and was analogous to the "Long Branch" clade of Fiedler *et al.* (2010) and the "Cosmopolitan" clade of Baeza (2010) (Fig. 4).

There was little support for the "Short Branch" clade *sensu* Fiedler *et al.* (2010). A number of species lacking a multi-article accessory branch (*L. amboinensis*, *L. ankeri* Rhyne & Lin, 2006, *L. boggessi* Rhyne & Lin, 2006, *L. bahia*, *L. debelius*, *L. grabhami*, *L. pederseni* Rhyne & Lin, 2006, *L. rafa* Rhyne & Anker, 2007, and *L. wurdemanni*), the species described as possessing a variable dorsal antennule (i.e., uniramous or with 1 or 2 articles; *L. californica*, *L. gracilirostris*, and *L. nayaritensis*), and *L. arvoredensis*, which possesses an unguiform accessory branch formed a poorly supported clade (p*P* = 57; ML < 50). Further reducing the support for a natural clade of the *Lysmata* species lacking a multi-article accessory branch was the uncertain basal position of *L. olavoi* and placement of *L. vittata* sensu Bruce (1990) in the "Morpho-variable" clade of Baeza (2010; Fig. 4).

There was also little support for a "Specialized Cleaner Shrimp" clade of Fiedler *et al.* (2010) and "Cleaner" clade of Baeza (2010), as *L. amboinensis*, *L. debelius*, and *L. grabhami* were recovered in a poorly supported clade (p*P* = 53; ML = < 50). The third strongly supported clade consisted of species in the greater *L. wurdemanni* species complex (*L. ankeri*, *L. boggessi*, *L. pederseni*, *L. rafa*, and *L. wurdemanni*) from the Caribbean/Gulf of Mexico/western Atlantic and *L. gracilirostris* from the eastern Pacific, which was analogous to the "Tropical American" clade of Baeza (2010) (Fig. 4).

DISCUSSION

Lysmata vittata *is a species complex*

Our analyses clearly indicate that *L. vittata* sensu lato represents a species complex of two morphologically and genetically distinct groups, containing at least three species. The first group agreed with *L. vittata* sensu Bruce (1990), as well as the brief original description of Stimpson (1860), most notably in that the dorsal antennule was uniramous, lacking an accessory branch, and included shrimp collected in Hong Kong, New Zealand, Taiwan, and the United States. This group formed a strongly supported clade (Fig. 4), which was delineated as a putative species in the ABGD analysis. It is noteworthy that the "Bruce Type" clade was most similar to the non-*Lysmata* allies included in our analysis: *L. prima* (*p* distance = 0.125), *E. ensirostris* (*p* distance = 0.122), and *M. heterocarpoides* (*p* distance = 0.129). These genetic distances were lower in comparison to members of the second group (*p* distance = 0.186–0.204), broadly called the "Rauli Type" clade, which was more similar to the description of *L. rauli* (currently regarded, as discussed above, as a junior synonym of *L. vittata*; Soledade *et al.*, 2013), as all examined members possessed a one-article accessory branch.

The "Rauli Type" group comprised two strongly supported sister clades: 1) "Northern Indo-West Pacific Rauli Type" clade, which included individuals from Brazil (the type locality of *L. rauli*), Hong Kong, and Thailand, and 2) "Southern Indo-West Pacific Rauli Type" clade, which only included individuals from Australia. Genetic differences (*p* distances) between the two "Rauli Type" clades were considerable (0.093–0.104) and larger than a number of interspecific comparisons within *Lysmata* and *Merguia*. Both clades were delineated as putative species at all partition levels in the ABGD analyses, which strongly suggests that the two "Rauli Type" clades represent distinct species in a geographically wide-ranging complex. While a more detailed morphological (including comparisons of live coloration) and genetic analysis is needed to determine species status and identify all

relevant diagnostic characters, our limited morphological examinations suggest there may be a difference in the number of second pereopod carpal articles, with shrimp from Australia having slightly higher (albeit overlapping) counts (17–26) in comparison to *L. vittata*/*L. rauli* from Brazil (15–19; Laubenheimer & Rhyne, 2010; Soledad *et al.*, 2013).

Our analyses, in combination with previous studies, suggest there are other taxonomically relevant differences in addition to accessory branch structure between *L. vittata* sensu Bruce (1990) and shrimp that share affinity with *L. rauli*, which further supports their recognition as separate evolutionary divergent groups. The most conspicuous difference is coloration, which, as noted, is an important diagnostic character within *Lysmata* (Rhyne & Lin, 2006; Rhyne *et al.*, 2012; Baeza & Behringer, 2017). The abdomens of all North American *L. vittata* possessed numerous thin red stripes without transverse bars, similar to *L. vittata* from Japan (Hayashi & Miyake, 1968) and southeastern Russia (Marin *et al.*, 2012). Conversely, our *L. rauli* from Hong Kong possessed red transverse bars at the anterior edge of the first somite and between the third and fourth somites. This pattern is similar to the holotype of *L. rauli* (Laubenheimer & Rhyne, 2010) and *L. vittata* collected in Brazil (Alves *et al.*, 2018, 2019), as well as *L. vittata* reported from Australia (Barton *et al.*, 2020), India (Kemp, 1914), Caribbean Panama (Pachelle *et al.*, 2018), Singapore (Anker & De Grave, 2016), and Egypt (Abdlesalam, 2018).

There also appear to be differences in the number of the carpal articles of the second pereopod, another important diagnostic character in *Lysmata* (see Chace, 1997). Although there was some overlap, the North American and New Zealand *L. vittata* and the topotypic material of Bruce (1990) examined herein (a subset of which comprised part of our "Bruce Type" clade) possessed a greater number of second pereopod carpal articles (18–23) in relation to most *L. rauli*/*L.* "*vittata*" from Brazil (15–19; Laubenheimer & Rhyne, 2010; Soledade *et al.*, 2013; Alves *et al.* 2018). Pachelle *et al.* (2020) reported a similar number of carpal articles for *L. vittata* from Brazil (15–19), except for a single individual (MZUSP 31541) with a carpal count of 22 to 23 (and a meral count of 12 to 13). This count was considerably higher than previous reports for any Brazilian "*L. vittata*" and warrants reexamination to confirm identification. The rostrum of the same North American and New Zealand *L. vittata* and the topotypic material of Bruce (1990) were also longer (reaching the distal margin of the second article of the antennular peduncle) in comparison to *L. rauli*/*L.* "*vittata*" from Brazil (reaching the midpoint of the second article of the antennular peduncle; Laubenheimer & Rhyne, 2010; Soledade *et al.*, 2013). It is noteworthy that our Australian *L.* cf. *rauli* were similar to *L. rauli*/*L. vittata* from Brazil in most respects, except for higher second pereopod carpal articles count (17–26) and slightly longer rostrum, which was more similar to our North American *L. vittata*. Caution should be taken when comparing small differences in rostral length, as it can be variable among *Lysmata* species and prone to damage or deformity.

Although not examined by us, limited data further indicate there may be geographic differences in larval morphology, specifically in relation to the rostrum length of the Zoea I and the number of aesthetascs and setae on the outer flagellum of the antennule of the Zoea III between individuals collected in India and Korea/southeastern Russia (Pillai, 1966; Yang & Kim, 2010; Marin *et al.*, 2012). Adult *L. vittata* from southeastern Russia collected concurrently to these zoeas shared a similar coloration pattern to our North American material (Marin *et al.*, 2012), whereas adults collected in India are reported to share a coloration pattern similar to *L. rauli* (Kemp, 1914).

We agree with Anker & De Grave (2016) and Pachelle *et al.* (2018), who surmised *L. vittata* comprised a species complex, given the notable variation in morphological characters across a large geographical distribution. Variability in important diagnostic characters, including the number of second pereopod carpal articles and accessory branch structure, reflect interspecific differences among the constituent entities rather than an extraordinarily wide intraspecific variation. Historically, *L. vittata* may have served as a "catch all" for Indo-West Pacific peppermint shrimps with numerous fine stripes lacking a multi-article accessory branch and possessing a pterygostomial spine and rostrum of intermediate length. Future studies should provide detailed morphological data and photographs whenever possible to avoid misidentifications. We predict that a review of *L. vittata* museum holdings will yield misidentified and further unrecognized species in this complex. As an example, Wang & Shu (2018) reviewed a large number of Chinese *L. vittata* museum specimens and noted variability in rostrum length (overreaching distal margin of second article of antennular peduncle to near the distal end of third article), first pereopod chelae shape (cutting surface flat when closed or with a gap proximally) and number of second pereopod carpal articles (18–27), and a re-examination may uncover the presence of *L. vittata* sensu Bruce (1990), *L. rauli*, or other *Lysmata* species.

The existence of a *L. vittata* species complex and the taxonomic importance of coloration patterns in delimiting *Lysmata* species are highlighted by the small number of *L. vittata* in Australian museums that we examined, which comprises at least three separate species. While the coloration pattern of *L. dispar* has not been reported, we assume it to be similar to *L. lipkei* (a possible junior synonym), which consists of three thick reddish stripes on the abdomen (Okuno & Fiedler, 2010; Pachelle *et al.*, 2016). If so, *L. dispar* and *L. vittata* are easily differentiated in life. Our Australian *L. dispar* were initially identified after preservation in ethanol, which quickly dissolves red pigments. Notwithstanding that *L. dispar* and *L. lipkei* were not yet described at the time of initial identification of the MAGNT material herein, the absence of any coloration was almost certainly a factor in the misidentification of *L. vittata*, in addition to inadequate morphological criteria.

Conflicting accessory branch morphological terminology confounds the taxonomy of Lysmata

Long-standing, conflicting and imprecise terminology regarding the status and structure of the accessory branch has confounded taxonomical and morphological assessments of the species of *Lysmata*. We support a rigorous morphological review of Lysmatidae and the creation of standardized accessory branch terminology, which may provide a clearer understanding of taxonomical differences and possibly better elucidate evolutionary relationships within *Lysmata* and Lysmatidae. Firstly, what specifically constitutes an accessory branch should be defined, taking care to reevaluate the accuracy of ambiguous terms, such as "vestigial" or "rudimentary" and how these terms have been applied across taxa. Secondly, we recommend revaluating descriptions of the shape and structure of the accessory branch, as variability among species/groups may be greater than currently conceived and may yet hold taxonomical significance. For example, there may be meaningful differences among the species reported to possess an unguiform or "claw-shaped" accessory branch. We found that the accessory branches of the *L. rauli* type material, *L.* cf. *rauli*, as well as *L. dispar,* were rounded and stump-like in comparison to the more elongated, pointed (and arguably more claw shaped) accessory branch of *L. arvoredensis*/*L. uncicornis* as shown in Holthuis & Maurin (1952), Giraldes *et al.* (2018), and González-Ortegón *et al.* (2020). We demur from suggesting a revised accessory branch terminology at this time pending a more thorough review of multiple lysmatid taxa.

The uncertainty regarding the *Lysmata* accessory branch is not unreasonable, as it can be minute, requiring high-level magnification to properly examine its shape and structure. The small size of the accessory branch of some shrimps in the broader *L. vittata* complex (e.g., those resembling *L. rauli*) is likely the reason this diagnostic character received little notice, even in such a widely

reported species group. Smaller accessory branches can be obscured by aesthetascs, and antennal articles containing aesthetascs are often thickened; thus, the last antennule article possessing an aesthetasc may protrude, possibly giving the false appearance of an accessory branch or some type of "free article." Published illustrations of the antennules often omit aesthetascs, which may affect accurate comparisons with fresh material. Literature that includes detailed photographs or illustrations can be helpful in properly accessing accessory branch structure.

We found no support for the *Hippolysmata* versus *Lysmata* split as historically conceived. Shrimps with a multi-article accessory branch formed a strong clade in both the BI and ML analyses, which included the type species of *Lysmata*, *L. seticaudata*, suggesting they form a natural group and may constitute *Lysmata* sensu stricto. *Lysmata* lacking a multi-article accessory branch were included in clades corresponding to the "Morpho-variable" clade of Baeza (2010) and the "Short Branch" clade of Fiedler *et al.* (2010) (Fig. 4), in addition to the uncertain placement of *L. olavoi*. Although the apparent homoplasy of a non-multi-article accessory branch certainly undermines the recognition of both *Lysmata* and *Hippolysmata* as valid monophyletic genera or subgenera, a rigorous comparison of the accessory branch structure across Lysmatidae will likely aid in refining our understanding of evolutionary relationships, particularly as current morphological classification criteria may be inadequate. If *Lysmata* is restricted to shrimps possessing a multi-article accessory branch, the genus *Hippolysmata* would not be available to any of the species once included in *Hippolysmata*, with the exception of *L. vittata* (the type taxon) given the phylogenetic placement of the *L. vittata* in the "Morpho-variable" clade. A revision of the genus incorporating molecular, morphological, and larval developmental data, which also includes related genera (e.g., *Exhippolysmata*, *Lysmatella*, *Mimocaris*) is required to make this system phylogenetically sound (Baeza, 2010). Counter to Baeza (2010) and Baeza & Fuentes (2013), our analyses found weak support for a "Specialized Cleaner Shrimp" clade, given the low node support values in the BI and ML analyses, though our data matrix (number of aligned nucleotides) was smaller than used in those studies. A more thorough analysis incorporating additional gene markers and sequences, including those of *L. splendida* may better elucidate interspecific relationships within this group and among other *Lysmata*.

Both major groups in the broader *L. vittata* species complex (*L. vittata* sensu Bruce (1990) and *L rauli*), in addition to *E. ensirostris*, *L. dispar*, *L. hochi*, *L. lipkei*, *L. prima*, *M. heterocarpoides*, and the unidentified Australian *Lysmata* species were strongly recovered in the "Morpho-variable" clade. This clade included all the *Lysmata* species with a short, rounded unguiform accessory branch included our analyses (*L. dispar*, *L. hochi*, *L. lipkei*, *L. rauli*, and *L.* cf. *rauli*). The position of these four species suggests they are basal to the other members of the "Morpho-variable" clade. Molecular data from other species with a one-article accessory branch (e.g., *L. anchisteus* Chace, 1972, *L. guamensis* Anker & Cox, 2011, *L. kuekenthali* De Man, 1902, *L. morelandi*, *L. multiscissa*, *L. philippinensis* Chace, 1997, *L. stenolepis* Crosnier & Forest 1973) would help clarify morpho-taxonomical relationships. The inclusion of *Lysmata* allies *Exhippolysmata*, *Lysmatella*, *Mimocaris* in the "Morpho-variable" clade rendered *Lysmata* paraphyletic and may indicate current generic criteria based on a raised basal crest and rostrum exceeding carapace length (*Exhippolysmata*), abdominal segments with large median posterior spines and long rostrum exceeding carapace length (*Mimocaris*), and lack of epipods on pereopods 3–5 (*Lysmatella*) does not merit the recognition of separate genera Fiedler *et al.*, 2010. Although the gross morphologies vary widely, species in the "Morpho-variable" clade may share similar sperm ultramorphology (see Baeza, 2010), further supporting close evolutionary relationships among species in this clade. A rigorous integrative taxonomic analysis incorporating denser taxon sampling, multiple genes, and developmental/reproductive data is needed to adequately revise *Lysmata* and resolve relationships within Lysmatidae.

Designation of a neotype for L. vittata Stimpson,1860

The type material for *L. vittata* was destroyed in the Great Chicago Fire of 1871 and no syntypes have been located in other museums (see Baldinger, 1998 and Evans, 1967). In order to delimit the putative *L. vittata* species complex, a neotype must be assigned. This neotype should reflect what Stimpson (1860) originally collected from the type locality as much as possible. Confounding the designation of a neotype are the two apparent morphotypes within the *L. vittata* complex, *L. vittata* sensu Bruce (1990) and *L. rauli*, both of which have been recently collected in Hong Kong (the type locality). In the absence of additional information regarding what Stimpson (1860) originally collected, the specimen from Hong Kong examined here and used by Bruce (1990) to redescribe *L. vittata* is a suitable candidate for selection as neotype. In Bruce's (1990) specimen, the dorsal antennule is uniramous and closely matches Stimpson's (1860) original description. This feature was a primary determinant in Stimpson (1860) creating a new genus *Hippolysmata* (for *L. vittata*), distinct from the earlier described *Lysmata*, whose members possess a multi-article accessory branch. Although admittedly not very detailed, the descriptions of color pattern for *L. vittata* provided by Bruce (1990: 608), which stated "numerous fine red longitudinal striae," and Stimpson (1860: 26), which stated "corpus coccineo-vittatum" ("scarlet-striped body") did not mention dark transverse bars, an apparent characteristic of *L. rauli*. Given the current ambiguity in identity of *L. vittata* (and consequent taxonomic instability caused to *L. rauli*), heterogeneity in the current understanding of *L. vittata*, and the presence of two *L. vittata*-like species at the type locality, clarification of the identity of the species is required. We herein designate the specimen figured and described by Bruce (1990: figs. 23–28) (ovigerous female, post-orbital carapace length 7.3 mm, Mirs Bay, Hong Kong; NTM Cr004983) as the neotype of *L. vittata* to fix the identity of the species. Bruce (1990) provided a detailed description and extensive figures of the specimen.

Unresolved taxonomy confounds delimiting the L. vittata *and* L. dispar/L. lipkei *species complexes*

With the present fixation of the neotype, specimens collected in the Chesapeake Bay region, New Zealand, and Taiwan that comprised our "Bruce Type" clade represent *L. vittata* sensu stricto. We further hypothesize that based on similar morphological descriptions and/or coloration patterns, *L. vittata* from Japan in Hayashi & Miyake (1968) and southeastern Russia in Marin *et al.* (2012) likely represent *L. vittata* sensu stricto. Although data are limited, these locations are all in temperate and subtropical regions, which may reflect habitat preferences or ecological constraints for *L. vittata* sensu stricto. With the Bruce (1990) material designated as the neotype, the common name often associated with *L. vittata*, the "Indian lined shrimp" (ITIS 2021; WoRMS 2021b), is rendered a misnomer, as *L. vittata* sensu stricto may not occur in India, and presumably may be more suitably applied to *L. rauli*. We suggest replacing "Indian Lined Shrimp" with the more apt "East-Asian Lined Shrimp" as the common name for *L. vittata* sensu stricto. Despite fixation of the neotype for *L. vittata*, the status of some the junior synonyms remains uncertain until fully revised, since, as noted above, the type material of *N. unirecedens* is in poor condition and the type material for *H. vittata* var. *subtillis* was destroyed. The neotype fixation of *L. vittata*, nevertheless, now provides a firm basis on which these nominal synonyms can be revised.

The type material (comprising three individuals) of *H. durbanensis* (NHM 1928.12.1.433) is available and a detailed reexamination is warranted, in addition to any supplemental material from the

region, though collections of *H. durbanensis*/*L. vittata* from southern Africa appear rather limited (Barnard, 1950). Further stressing the need to review the status of *H. durbanensis* are several differences noted in the original description (Stebbing, 1921) and between the type material of *H. durbanensis* and *L. vittata* from the Indo-West Pacific and western Atlantic, which may hold taxonomic significance. These include the color of eggs (white in *H. durbanensis*, green/yellow in *L. vittata*), the shape of the major chelae (fingers that close together or with a very narrow gap in *H. durbanensis*, but a wide gap proximally in *L. vittata*), the number of ventral spines on the dactyls of pereopods 3–5 (three to four in *H. durbanensis*, five to six in *L. vittata*), and the relatively longer rostrum (exceeding the antennular peduncle) and shorter antennal scale (not exceeding the second article of the antennular peduncle) as inferred from the illustrations in Stebbing (1921). It should be noted that Stebbing (1921: 20) stated that individuals of *H. durbanensis* were obtained "along with many other species cast on the beach of Durban Bay." This may indicate they were dead for an unknown period of time before collection, which may have affected egg coloration.

Given the fixation of the "Bruce Type" clade as *L. vittata* sensu stricto, our data support the resurrection of *L. rauli* to a full species. Resolving the status of *L. rauli*, however, is confounded, as our phylogenetic analyses also suggest *L. rauli* may itself be a complex of two sister species. Fully delimiting the putative *L. rauli* species complex requires further detailed analyses and was beyond the scope of this investigation. As the type locality for *L. rauli* is Brazil, nevertheless, our "Northern Indo-West Pacific Rauli Type" clade, which contained individuals from Brazil, Thailand, and Hong Kong, likely represents *L. rauli* sensu stricto, native to the Indo-West Pacific. The description of *L. rauli* from Brazil in this sense would reflect a well-known pattern of many introduced species being first described in their non-native range (Carlton, 2009).

Our "Southern Indo-West Pacific Rauli Type" clade, which contained individuals from northern Australia, likely represents an undescribed species. Based solely on coloration it is difficult to classify shrimps from other studies that resemble *L. rauli* according to the geographic clades reported herein (i.e., "Northern and Southern Indo-West Pacific Rauli Type" clades), as individuals from Australia (Barton *et al.*, 2020) and Brazil (Laubenheimer & Rhyne, 2010; Soledade *et al.*, 2013; Alves *et al.*, 2018, 2019) appear similar (i.e., both possess transverse bands). We presume the "*L. vittata*" reported from the Caribbean Sea coast of Panama by Pachelle *et al.* (2018) are similar to those collected further south in Brazil (i.e., our "Northern Indo-West Pacific Rauli Type" clade). The "*L. vittata*" from the eastern Mediterranean (Abdlesalam, 2018) likely resulted from Lessepsian migration, particularly as the rate of introduction of non-native species into the Mediterranean from the Red Sea has increased since widening of the Suez Canal (Castellanos-Galindo *et al.*, 2020). As such, Mediterranean "*L. vittata*" could share affinity with our "Northern Indo-West Pacific Rauli Type" clade. A rigorous range-wide integrative taxonomic analysis is needed to determine the range of the different species in the putative *L. rauli* complex and the identity of introduced populations. This need is further highlighted as the ranges of both putative species may overlap to some extent in the Indo-West Pacific region.

Although we found evidence of geographic population structuring (i.e., Japan, Brazil, and Australia) for *L. dispar*/*L. lipkei*, both species were recovered in a strongly supported clade, which was recovered as a single putative species in the initial partition of the ABGD analysis. The genetic distances within this clade were much lower (maximum p distance = 0.025) than distances between other *Lysmata* species (e.g., minimum interspecific p distance = 0.043 in *L. grabhami* and *L. amboinensis*). In combination with the general ambiguity of the diagnostic characters that delineate *L. dispar* and *L. lipkei*, though limited, these data suggest they may represent a single wide-ranging species, with L. *lipkei* as a junior synonym of *L. dispar*. Pachelle *et al.* (2016) verified the first non-native introduction of *L. lipkei* by comparing shrimp collected in Brazil with a paratype of *L. lipkei* from Japan and reported no clear differences in morphology or live coloration patterns. Similar to our morphological examinations of Australian *L. dispar*, Pachelle *et al.* (2016) also noted morphological discrepancies in the Brazilian material. Namely, the articulations of the second pereopod ischium were clearly visible as in *L. dispar* and with our Australian material. Both the Brazilian *L. lipkei* and our Australian *L. dispar* possessed a minute spinule in the distal article of the antennular peduncle as in *L. lipkei*, an important diagnostic difference between both species as reported by Okuno & Fiedler (2010). Hayashi (2007) nevertheless does not explicitly state *L. dispar* lacks this spinule, but rather it is not illustrated (although spinules on the first and second articles are illustrated) nor specifically mentioned in the text. An integrative taxonomic examination of *L. dispar* and *L. lipkei* from across their putative native and introduced ranges, including comparisons of the type material is needed to fully establish the status of both species. Our report of *L dispar* is the second in the literature, and extends the known range from the type locality of the Dampier Archipelago, Western Australia to Heron Island, Queensland. *L. lipkei* has been reported from Japan (type locality), Singapore, Brazil, and most recently in southern Madagascar (Okuno & Fiedler, 2010; Anker & De Grave, 2016; Pachelle *et al.*, 2016; Ashrafi *et al.*, 2021).

Introduction of L. vittata *in the Chesapeake Bay region and New Zealand, and* L. californica *in New Zealand*

We document the first introduction of *L. vittata* in North America. The Chesapeake Bay region has been sampled extensively for over two centuries and given the conspicuous appearance of *L. vittata*, it is exceedingly unlikely this shrimp was simply overlooked, even considering its superficial similarity to *L. wurdemanni*. Individuals of *L. vittata* were often caught together with *L. wurdemanni*. Peppermint shrimps can be gregarious (Baeza, 2013), both with conspecifics and (occasionally) with congeners, and it is not surprising that these two species might co-occur. We are uncertain of what impact *L. vittata* may have on *L. wurdermanni* populations or other native species and further investigations are needed to determine any negative effects on ecosystem function due to this recent introduction.

The Chesapeake Bay *L. vittata* was almost certainly introduced via vessels arriving from the Western Pacific, likely via ballast water, though hull fouling is a possibility. Ballast water has been proposed as the probable vector for the introduction of the Asian caridean shrimp *Palaemon modestus* Heller, 1862 along the U.S. Pacific coast (Emmett *et al.*, 2002), the Ponto-Caspian mysid *Hemimysis anomala* Sars, 1907 in the Great Lakes (Walsh *et al.*, 2010), and the Asian shrimp *Palaemon macrodactylus* Rathbun, 1902 and the Asian shore crab *Hemigrapsus sanguineus* De Haan, 1835 (in De Haan, 1835–1850) (Fofonoff *et al.*, 2021). The lower Chesapeake Bay is home to numerous high volume commercial ports, such as Newport News Terminal, Port Norfolk, as well the largest naval station in the world in Norfolk, Virginia. While lysmatid shrimps are popular in the aquarium trade due to their attractive appearance, fish "cleaning" behavior (Vaughan, *et al.*, 2016), and ability to control nuisance sea anemones (e.g., *Aiptasia* spp.; Rhyne *et al.*, 2004) and *Acropora*-eating flatworms (Barton *et al.*, 2020) via predation, aquarium releases were unlikely to be the introduction vector for *L. vittata*. Unless specially ordered, *L. vittata* does not appear readily available in the region's aquarium stores (RA, personal observation), whereas the congeners *L. wurdemanni* and *L. boggessi* sourced from Florida are extremely common (Baeza & Behringer, 2017) and less expensive than other imported peppermint shrimps (RA, personal observation).

While we are also uncertain of the exact timing of the *L. vittata* introduction in the region, given shrimp were first collected from within the Chesapeake Bay proper (~42 km from the bay mouth) and in a coastal embayment (Burtons Bay ~ 75 km north of the

bay's mouth) within a few months' time, it was likely introduced sometime prior to 2013. A thorough review museum and voucher collections in the region may help to refine the introduction timeline.

Delimiting the *L. vittata* species complex and determining the ranges and habitat preferences of these species is critical in estimating potential distributional expansion and impacts on native ecosystems. For example, *L. vittata* sensu lato has been widely reported from tropical, semi-tropical, and temperate areas and it is likely that member species have more specific temperature tolerances; however, what we presume to be *L. vittata* sensu stricto has been predominantly noted in subtropical and temperate areas (e.g., Taiwan, Hong Kong, Japan, southeastern Russia, and northern New Zealand). Little is known about habitat preferences within the broader complex with most records occurring from shallow coastal areas (Chace, 1997; Marin *et al*., 2012; Anker & De Grave, 2016; Pachelle *et al*., 2018) with more limited data from estuarine areas (Alves *et al*., 2018). Determining the native ranges of the constituent members of the *L. vittata* complex is an important step in fully understanding invasion potential. Evidence from our field collections suggest *L. vittata* sensu stricto has the potential to spread widely along the mid-Atlantic, given its apparent ability to survive low winter temperatures in the lower Chesapeake Bay and adjacent coastal embayments (minimum collection temperature 4.48 °C), as well as the polyhaline conditions of lower estuaries (minimum collection salinity 18.48 psu). Individuals of *L. vittata* were captured over an array of substrate types (sand, mud, and subtidal oyster reef) and depths (0.5 to nearly 20 m). Although we did not collect any individuals of *L. vittata* from beds of submerged aquatic vegetation, sampling effort in these areas was much less than the other habitats listed above.

Similar to all species of *Lysmata* studied to date (Baeza, 2008, 2013), we presume *L. vittata* sensu stricto displays protandric simultaneous hermaphroditism (PSH). In PSH, young individuals first mature as males and later transition to a phenotypically female phase, but will retain male gonadal tissue and can mate as both males and females (Bauer, 2000). This sexual system may be favorable to small founder populations. The *L. vittata* reported from Korea (which we presume represents *L. vittata* sensu stricto) exhibited a relatively long planktonic larval period (27–45 d through nine zoeal stages; Yang & Kim, 2010), which may increase invasion potential at regional scales.

Our genetic analyses confirm the presence of *L. vittata* sensu stricto and *L. californica* in New Zealand. Ahyong (2010) suggested *L. vittata* was likely native and previously overlooked or confused with *L. morelandi*. This assessment was predicated on the assumption that *L. vittata* ranged throughout the Indo-West Pacific, including northern Australia. With recognition of the *L. vittata* species complex, however, our analyses indicate that previous records of *L. vittata* from northern Australia probably represent a suite of different species. We suggest that *L. vittata* in New Zealand is more likely to have been introduced. It is noteworthy that most specimens have been collected near ports (Ahyong, 2010; MBP, 2021), which could indicate shipping as an introduction vector, although this may be an artifact of sampling effort. While there are scant survey data for New Zealand *L. vittata* post-2009 (MBP, 2021), citizen science observations submitted to iNaturalist (https://www.inaturalist.org) of shrimp with coloration patterns resembling *L. vittata* sensu stricto from eastern Auckland (observation 33638133) and Waikato (observation 33997888) in September, 2019 and western Auckland (observation: 73551063) and eastern Auckland (observation 75753165) in March-April, 2021 (GBIF, 2021) suggest *L. vittata* may currently be present and established in New Zealand. Collections of *L. californica* have occurred throughout northern New Zealand from 2012 to 2020 (GBIF, 2021; MBP, 2021) indicating it is also established in New Zealand, which represents the first introduction of this species. The native range of *L. californica* comprises coastal areas of the eastern Pacific from southern California to northern Mexico, including the Gulf of California and likely arrived in New Zealand due to ballast water/shipping. As *L. californica* is a cool-water species, it is not as common in the aquarium trade as tropical *Lysmata* species (see Calado (2020); RA, personal observation), and is not a permitted import in New Zealand (MPI, 2017). A directed quantitative sampling effort is warranted to determine population status and distribution of non-native *Lysmata* in New Zealand.

The recognition of *L. vittata* as a species complex has highlighted the importance and continued relevance of classical taxonomic/morphological investigation. *Lysmata vittata* sensu lato does not represent a "cryptic" complex of species with near identical morphologies (though this may be more of the case in *L. rauli*). Rather, it is a set of divergent species that while superficially similar, show clear and noticeable morphological differences upon inspection. It remained uncovered due to a lack of thorough investigation, confounded by unresolved issues of taxonomic nomenclature and terminology. It was not hidden, but rather hiding in plain sight.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. *Lysmata* species and other selected hippolytid and merguiid shrimps used in phylogenetic analyses

S2 Table. List of collection locations of *Lysmata vittata* sensu stricto in the greater Chesapeake Bay region.

S3 Table. Interspecific and intraspecific pairwise distances at 16S rRNA gene among *Lysmata* species and other selected hippolytid and merguiid shrimps.

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REFERENCES

Abdlesalam, K. 2018. First record of exotic Lysmatid shrimp *Lysmata vittata* (Stimpson, 1860) (Decapoda: Caridea: Lysmatidae) from the Egyptian Mediterranean coast. *Mediterranean Marine Science*, **19**: 124–131.

Aguilar, R., Ogburn, M.B., Driskell, A.C., Weigt, L.A., Groves, M.C. & Hines, A.H. 2017. Gutsy genetics: identification of digested piscine prey items in the stomach contents of sympatric native and introduced warmwater catfishes via DNA barcoding. *Environmental Biology of Fishes*, **100**: 325–336.

Ahyong, S.T. 2010. New species and new records of Caridea (Hippolytidae: Pasiphaeidae) from New Zealand. *Zootaxa*, **2372**: 341–357.

Albano, P.G., Steger, J., Bakker, P.A.J., Bogi, C., Bošnjak, M., Guy-Haim, T., Huseyinoglu, M.F., LaFollette, P.I., Lubinevsky, H., Mulas, M., Stockinger, M., Azzarone, M. & Sabelli, B. 2021. Numerous new records of tropical non-indigenous species in the Eastern Mediterranean highlight the challenges of their recognition and identification. *ZooKeys*, **1010**: 1–95.

Almeida, A.S., Barros-Alves, S.P., Hirose, G.L. & Alves, D.F.R. 2018. Reproductive output of the ornamental shrimp *Lysmata vittata* (Stimpson, 1860) (Decapoda: Caridea) in wild populations and under different maturation diets, *Invertebrate Reproduction & Development*, **62**: 257–267.

Ashrafi, H., Baeza, J.A. & Ďuriš, Z. 2021. The caridean shrimps of the genus *Lysmata* Risso, 1816 (Decapoda: Lysmatidae) from Madagascar collected during the Atimo-Vatae expedition: a new species and two new records. *European Journal of Taxonomy*, **774**: 155–177.

Alves, D.F.R., Lima, D.J.M., Hirose, G.L., Martinez, P.A., Dolabella, S.S. & Barros-Alves, S.P. 2018. Morphological and molecular analyses confirm the occurrence of two sympatric *Lysmata* shrimp (Crustacea, Decapoda) in the southwestern Atlantic. *Zootaxa*, **4526**: 41–55.

Alves, D.F.R., López Greco, L.S., Barros-Alves, S.P. & Hirose, G.L. 2019. Sexual system, reproductive cycle and embryonic development of the red-striped shrimp *Lysmata vittata*, an invader in the western Atlantic Ocean. *PLoS ONE*, **14**: e0210723 [https://doi.org//10.1371/journal.pone.0210723].

Anker, A. & Cox, D. 2011. A new species of the shrimp genus *Lysmata* Risso, 1816 (Crustacea, Decapoda) from Guam. *Micronesica*, **41**: 197–214.

Anker, A. & De Grave, S. 2016. An updated and annotated checklist of marine and brackish caridean shrimps of Singapore (Crustacea, Decapoda). *Raffles Bulletin of Zoology*, **2016**: 343–454.

Baeza, J. 2008. Protandric simultaneous hermaphroditism in the shrimps *Lysmata bahia* and *Lysmata intermedia*. *Invertebrate Biology*, **127**: 181–188.

Baeza, J.A. 2010. Molecular systematics of peppermint and cleaner shrimps: Phylogeny and taxonomy of the genera *Lysmata* and *Exhippolysmata* (Crustacea: Caridea: Hippolytidae). *Zoological Journal of the Linnean Society*, **160**: 254–265.

Baeza, J.A. 2013. Molecular phylogeny of broken-back shrimps (genus *Lysmata* and allies): A test of the 'Tomlinson-Ghiselin' hypothesis explaining the evolution of hermaphroditism. *Molecular Phylogenetics and Evolution*, **69**: 46–62.

Baeza, J.A. & Anker, A. 2008. *Lysmata hochi* n. sp., a new hermaphroditic shrimp from the southwestern Caribbean Sea (Caridea: Hippolytidae). *Journal of Crustacean Biology*, **28**: 148–155.

Baeza, J.A. & Behringer, D.C. 2017. Integrative taxonomy of the ornamental 'peppermint' shrimp public market and population genetics of *Lysmata boggessi*, the most heavily traded species worldwide. *PeerJ*, **5**: e3786 [https://doi.org/10.7717/peerj.3786].

Baeza, J.A. & Fuentes, M.S. 2013. Exploring phylogenetic informativeness and nuclear copies of mitochondrial DNA (Numts) in three commonly used mitochondrial genes: Mitochondrial phylogeny of peppermint, cleaner, and semi-terrestrial shrimps (Caridea: *Lysmata*, *Exhippolysmata*, and *Merguia*). *Zoological Journal of the Linnean Society*, **168**: 699–722.

Baeza, J.A., Bolaños, J.A., Hernández, J.E. & López, R. 2009a. A new species of *Lysmata* (Crustacea, Decapoda, Hippolytidae) from Venezuela, southeastern Caribbean Sea. *Zootaxa*, **2240**: 60–66.

Baeza, J.A., Schubart, C.D., Zillner, P., Fuentes, S. & Bauer, R.T. 2009b. Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of protandric simultaneous hermaphroditism and social monogamy. *Biological Journal of the Linnean Society*, **96**: 415–424.

Baldinger, A.J. 1998. The crustacean collection at the Museum of Comparative Zoology, Harvard University. In: *Crustaceans and the Biodiversity Crisis, The Proceedings of the fourth International Crustacean Congress*, **Vol. 1** (F.R. Schram & J.C. von Vaupel Klein, eds.), pp. 45–59. Brill, Leiden, The Netherlands.

Barnard, K.H. 1950. Descriptive catalogue of South African decapod Crustacea (crabs and shrimps). *Annals of the South African Museum*, **38**: 1–864.

Barton, J.A., Humphrey, C., Bourne, D.G. & Hutson, K.S. 2020. Biological controls to manage *Acropora*-eating flatworms in coral aquaculture. *Aquaculture Environment Interactions*, **12**: 61–66.

Bauer, R.T. 2000. Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. *Journal of Crustacean Biology*, **20**(Special Number 2): 116–128.

Bax, N., Williamson, A., Aguero, M., Gonzalez, E. & Geeves, W. 2003. Marine invasive alien species: a threat to global biodiversity. *Marine Policy*, **27**: 313–323.

Borradaile, L.A. 1915. Notes on Carides. *Annals and Magazine of Natural History*, Series 8, **15**(8): 205–213.

Bruce, A.J. 1983. *Lysmata debelius* new species, a new hippolytid shrimp from the Philippines. *Revue française d'Aquariologie*, **9**: 115–120.

Bruce, A.J. 1990. Redescriptions of five Hong Kong carideans first described by William Stimpson, 1860. In: *Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China*. (B. Morton, ed.), pp. 569–610. Hong Kong University Press, Hong Kong.

Burukovsky, R.N. 2000. *Lysmata splendida* sp. nov., a new species of shrimp from the Maldives (Crustacea: Decapoda: Hippolytidae). *Senckenbergiana maritima*, **30**: 223–227.

Calado, R. 2020. Marine ornamental decapods—Collection, culture, and conservation. In: *Fisheries and Aquaculture*, **Vol. 9** (G. Lovrich & M. Thiel, eds.), pp. 313–340. Oxford University Press, New York.

Carlton, J.T. 2009. Deep invasion ecology and the assembly of communities in historical time. In: *Biological invasions in marine ecosystems: ecological, management, and geographic perspectives* (G. Rilov & J. A. Crooks, eds.), pp. 13–56. Springer-Verlag, Berlin.

Castellanos-Galindo, G.A., Robertson, D.R. & Torchin, M.E. 2020. A new wave of marine fish invasions through the Panama and Suez canals. *Nature Ecology and Evolution*, **4**: 1444–1446.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**: 540–552.

Chace, F.A. 1970. A new shrimp of the genus *Lysmata* (Decapoda, Hippolytidae) from the western Atlantic. *Crustaceana*, **19**: 59–66.

Chace, F.A. 1972. The shrimps of the Smithsonian-Bredin Caribbean expeditions with a summary of the West Indian shallow-water species (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology*, **98**: 1–179.

Chace, F.A. 1997. The caridean shrimps (Crustacea: Decapoda) of the Albatross Philippine Expedition, 1907–1910, Part 7: Families Atyidae, Eugonatonotidae, Rhynchocinetidae, Bathypalaemonellidae, Processidae, and Hippolytidae. *Smithsonian Contributions to Zoology*, **587**: 1–106.

Crosnier, A. & Forest, J. 1973. Les crevettes profondes de l'Atlantique Oriental Tropical. *Faune Tropicale*, **19**: 1–409

Dana, J.W. 1852. Conspectus Crustaceorum, etc. Conspectus of the Crustacea of the Exploring Expedition under Capt Wilkes, U.S.N., including the Crustacea Cancroidea Corystoidea. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **6**: 10–28.

De Grave, S., Li, C.P., Tsang, L.M., Chu, K.H. & Chan, T.Y. 2014. Unweaving hippolytoid systematics (Crustacea, Decapoda, Hippolytidae): resurrection of several families. *Zoologica Scripta*, **43**: 496–507.

Dueñas, M.A., Ruffhead, H.J., Wakefield, N.H., Roberts, P.D., Hemming, D.J. & Diaz-Soltero, H. 2018. The role played by invasive

species in interactions with endangered and threatened species in the United States: a systematic review. *Biodiversity and Conservation*, **27**: 3171–3183.

Emmett, R.L., Hinton, S.A., Logan, D.J. & McCabe, G.T., Jr. 2002. Introduction of a Siberian freshwater shrimp to western North America. *Biological Invasions*, **4**: 447–450.

Evans, A.C. 1967. Syntypes of Decapoda described by William Stimpson and James Dana in the collections of the British Museum (Natural History). *Journal of Natural History*, **1**: 399–411.

Fiedler, G.C., Rhyne, A.L., Segawa, R., Aotsuka, T. & Schizas, N.V. 2010. The evolution of euhermaphroditism in caridean shrimps: a molecular perspective of sexual systems and systematics. *BMC Evolutionary Biology*, **10**: 297 [https://doi.org//10.1186/1471-2148-10-297].

Filipová, L., Holdich, D.M., Lesobre, J., Grandjean, F. & Petrusek, A. 2010. Cryptic diversity within the invasive virile crayfish *Orconectes virilis* (Hagen, 1870) species complex: new lineages recorded in both native and introduced ranges. *Biological Invasions*, **12**: 983–989.

Fofonoff, P.W., Ruiz, G.M., Steves, B., Simkanin, C. & Carlton, J.T. 2021. National Exotic Marine and Estuarine Species Information System [http://invasions.si.edu/nemesis/] (accessed 26 January 2021).

Froglia, C. & Deval, M.C. 2013. First record of an exotic hippolytid shrimp in the eastern Mediterranean Sea. *Mediterranean Marine Science*, **15**: 168–171.

Fransen, C.H.J.M. 1991. *Lysmata olavoi*, a new shrimp of the family Hippolytidae (Decapoda, Caridea) from the Eastern Atlantic Ocean. *Arquipelago*, **9**: 63–73.

Gan, Z. & Li, X. 2016. *Lysmata leptodactylus*, a new species of lysmatid shrimp (Crustacea: Decapoda: Caridea) from China. *Zootaxa*, **4138**: 181–188.

GBIF (Global Biodiversity Information Facility). 2021. [https://doi.org/10.15468/dl.z7uagg] (accessed 21 May 2021).

Gibbes, L.R. 1850. On the carcinological collections of the United States, and an enumeration of species contained in them, with notes on the most remarkable, and descriptions of new species. *Proceedings of the American Association for the Advancement of Science*, **3**: 165–201.

Giraldes, B.W., Macedo, T.P., Brandão, M.C., Baeza, J.A. & Freire, A.S. 2018. *Lysmata arvoredensis* nov. sp., a new species of shrimp from the south coast of Brazil with a key to species of *Lysmata* (Caridea: Lysmatidae) recorded in the southwestern Atlantic. *PeerJ*, **6**: e5561 [https://doi.org/10.7717/peerj.5561].

Gordon, I. 1935. On new and imperfectly known species of Crustacea Macrura. *Journal of the Linnean Society, Zoology*, **39**: 307–351.

González-Ortegón, E., García-Raso, J.E., Calado, R. López de la Rosa, I., Guerrero, M. & Cuesta, J.A. 2020. Atlantic expansion of the African caridean shrimp *Lysmata uncicornis* Holthuis & Maurin, 1952 (Caridea: Lysmatidae). *Marine Biodiversity*, **50**: 26.

Haan, W. de. 1833–1850. Crustacea. In: *Fauna Japonica sive Descriptio Animalium, quae in Itinere per Japoniam, Jußu et Auspiciis Superiorum, qui Summum in India Batava Imperium Tenent, Suscepto, Annis 1823-1830 Collegit, Notis, Observationibus et Adumbrationibus Illustravit* (P.F. von Siebold, ed.). Lugduni-Batavorum [= Leiden].

Hale, H.M. 1929. Notes on the fauna of Dirk Hartog Island, Western Australia: No. 4. Crustacea. *Transactions of the Royal Society of South Australia*, **53**: 67–70.

Hanamura, Y. 2008. A new species of *Eualus* Thallwitz, 1891 and a new record of *Lysmata morelandi* (Yaldwyn, 1971) (Decapoda, Caridea, Hippolytidae) from South-Eastern Australia. *Crustaceana*, **81**: 87–97.

Hayashi, K.I. 2007. *Lysmata dispar* sp. nov., a new shrimp from the Dampier Archipelago, Western Australia, with notes on other species of the family Hippolytidae (Crustacea: Decapoda: Caridea. *Records of the Western Australian Museum* Supplement, **73**: 85–95.

Hayashi, K.I. & Miyake, S. 1968. Studies on the hippolytid shrimps from Japan: V. Hippolytid fauna of the sea around Amakusa Marine Biological Laboratory. *Occasional Papers of Zoological Laboratory*, **1**: 121–163.

Heller, C. 1861. Beitrage zur Crustaceen-Fauna des rothen Meeres. Zweiter Theil. *Sitzungsberichte der Mathematisch-Naturwissenschaftlichen Classe der Kaiserlichen Academie der Wissenschaften*, **44**: 241–295.

Heller, C. 1862. Neue Crustaceen, gesammelt während der Weltumseglung der k.k. Fregatte Novara. Zweiter vorläufiger Bericht. *Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien*, **12**: 519–528.

Hirsch, H., Gallien, L., Impson, F.A.C., Kleinjan, C. Richardson, D.M. & Le Roux, J.J. 2017. Unresolved native range taxonomy complicates inferences in invasion ecology: *Biological Invasions*, **19**: 1715–1722.

Holthuis, L.B. & Maurin, C. 1952. Note sur *Lysmata uncicornis* nov. spec. et sur deux autres espèces intéressantes de crustacés décapodes macroures de la côte Atlantique du Maroc. *Proceedings van de Koninklijke Nederlandsche Akademie van Wetenschappen*, **55**: 197–202.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755.

ITIS (Integrated Taxonomic Information System). 2021. *Lysmata vittata* (Stimpson, 1860). [https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=96907#null] (accessed 2 March 2021).

Jose, S., Paramasivam, P., Madhavan, M., Sundaramoorthy, B. Dhina-Karan, A. Ajith Kumar, T.T. & Lal, K.K. 2020. Two new records of hippolytoid shrimps, *Lysmata hochi* Baeza & Anker, 2008 (Decapoda: Lysmatidae) and *Lysmata amboinensis* (De Man, 1888) from Lakshadweep Islands, India with taxonomic notes. *Zootaxa*, **4755**: 353–364.

Katoh, K. & Toh, H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics*, **9**: 286–298.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**: 3059–3066.

Kemp, S. 1914. Notes on Crustacea Decapoda in the Indian Museum: V. Hippolytidae. *Records of the Indian Museum*, **10**: 81–129.

Kensley, B.F. 1981. On the zoogeography of southern African decapod Crustacea, with a distributional checklist of the species. *Smithsonian Contributions to Zoology*, **338**: 1–64.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**: 111–120.

Kingsley, J.S. 1878. Notes on the North American Caridea in the Museum of the Peabody Academy of Science at Salem, Mass. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **1878**: 89–98.

Kubo, I. 1951. Some macrurous decapod Crustacea found in Japanese waters, with descriptions of four new species. *Journal of the Tokyo University of Fisheries*, **38**: 259–289.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**: 1870–1874.

Kumschick, S., Gaertner, M., Vilà, M., Essl, F., Jeschke, J.M., Pyšek, P., Ricciardi, A., Bacher, S., Blackburn, T.M., Dick, J.A.T., Evans, T. Hulme, P.E., Kühn, I., Mrugała, A., Pergl, J. Rabitsch, W., Richardson, D.M., Sendek, A. & Winter, M. 2015. Ecological impacts of alien species: quantification, scope, caveats, and recommendations, *BioScience*, **65**: 55–63.

Laubenheimer, H. & Rhyne, A.L. 2010. *Lysmata rauli*, a new species of peppermint shrimp (Decapoda: Hippolytidae) from southwestern Atlantic. *Zootaxa*, **2372**: 298–304.

Leray, M. & Knowlton, N. 2015. DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **112**: 2076–2081.

Man, J.G. de. 1888. Bericht über die von Herrn Dr. J. Brock im indischen Archipel gesammelten Decapoden und Stomatopoden. *Archiv für Naturgeschichte*, **53**(2): 215–600.

Man, J.G. de. 1902. Die von Herrn Professor Kükenthal im Indischen Archipel gesammelten Dekapoden und Stomatopoden. *In: W. Kükenthal Ergebnisse einer zoologischen Forschungsreise in den Molukken und Borneo, im Auftrage der Senckenbergischen naturforschenden Gesellschaft*, **25**: 465–929.

Man, J.G. de. 1907. On a collection of Crustacea, Decapoda and Stomatopoda, chiefly from the Inland Sea of Japan; with descriptions of new species. *Transactions of the Zoological Society of London*, **9**: 387–454.

Marin, I.N., Korn, O.M. & Kornienko, E.S. 2012. The caridean shrimp *Lysmata vittata* (Stimpson, 1860) (Decapoda: Hippolytidae): a new species for the fauna of Russia. *Russian Journal of Marine Biology*, **38**: 359–363.

Marchini, A. & Cardeccia, A. 2017. Alien amphipods in a sea of troubles: cryptogenic species, unresolved taxonomy and overlooked introductions. *Marine Biology*, **164**: 69 [https://doi.org/10.1007/s00227-017-3093-1].

MBP (Marine Biosecurity Porthole). 2021. Enhanced search for *Lysmata* [https://www.marinebiosecurity.org.nz/search-for-species] (accessed 20 May 2021).

Martínez-Arce, A., De Jesús-Navarrete, A. & Leasi, F. 2020. DNA barcoding for delimitation of putative Mexican marine nematodes species. *Diversity*, **12**: 107 [https://doi.org//10.3390/d12030107].

McLeish, J., Briers, R.A., Dodd, J.A. & Rueckert, S. 2020. First genetic evidence that invasive bullhead (*Cottus* L. 1758) in Scotland is of English origin and the difficulty of resolving the European *Cottus* species taxonomy. *Journal of Fish Biology*, **96**: 617–630.

MPI (Ministry for Primary Industries). 2017. *Import Health Standard: Ornamental Fish and Marine Invertebrates – Import Health Standard, ORNAMARI.ALL*. Wellington, New Zealand.

Molnar, J.L., Gamboa, R.L., Revenga, C. & Spalding, M.D. 2008. Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, **6**: 485–492.

Nobili, G. 1903. Contributo all fauna carcinologia di Borneo. *Bollettino dei Musei di Zoologia ed Anatomia comparata della Regia, Università di Torino*, **18**(447): 1–32.

Nobili, G., 1904. Diagnoses préliminaires de vingt-huit espèces nouvelles de stomatopodes et décapodes macroures de la Mer Rouge. *Bulletin du Muséum d'Histoire naturelle*, **10**: 228–238.

Okuno, J. & Fiedler, G.C. 2010. *Lysmata lipkei* a new species of peppermint shrimp (Decapoda: Hippolytidae) from warm temperate and subtropical waters of Japan. In: Studies on Malacostraca. Lipke Bijdeley Holthuis Memorial Volume (C.H.J.M. Fransen, S. De Grave, & P.K.L. Ng, eds.). *Crustaceana Monographs*: **14**: 597–610.

Pachelle, P.P., Anker, A., Mendes, C.B. & Bezerra, L.E. 2016. Decapod crustaceans from the state of Ceará, northeastern Brazil: an updated checklist of marine and estuarine species, with 23 new records. *Zootaxa*, **4131**: 1–63.

Pachelle, P.P.G., Carvalho, L., Alves, D.F.R. & Anker, A. 2020. A revision of the Brazilian species of *Lysmata* Risso, 1816 (Decapoda: Caridea: Lysmatidae), with discussion of the morphological characters used in their identification. *Zootaxa*, **4789**: 055–090.

Pachelle, P.P.G., Leray, M., Anker, A. & Lasley, R. 2018. Five new records of marine shrimps (Decapoda: Caridea, Stenopodidea) from the Caribbean coast of Panama. *Zootaxa*, **4438**: 128–136.

Palumbi, S.R., Martin, A.P., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. 1991. *A simple fool's guide to PCR, v2.0*. University of Hawaii and Kewalo Marine Laboratory, Honolulu, HI, USA.

Pillai, S.V. 1966. Some observations on the early larval stages of *Hippolysmata vittata* (Stimpson). *Journal of the Marine Biology Association of India*, **8**: 152–158.

Prakash, S. & Baeza, J.A. 2017. A new species of *Lysmata* Risso, 1816 (Crustacea, Decapoda, Lysmatidae) from the Gulf of Mexico. *Zootaxa*, **4363**: 576–582.

Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, **8**: 1864–1877.

Rathbun, M.J. 1900. Results of the Branner-Agassiz Expedition to Brazil. I. The decapod and stomatopod Crustacea. *Proceedings of the Washington Academy of Sciences*, **2**: 133–156.

Rathbun, M.J. 1901. Investigations of the aquatic resources and fisheries of Porto Rico by the United States Fish Commission Steamer Fish Hawk in 1899. The Brachyura and Macrura of Porto Rico. *Bulletin of the United States Fish Commission*, **20**: 1–127.

Rathbun, M.J. 1902. Japanese stalk-eyed crustaceans. *Proceedings of the United States National Museum*, **26**: 23–55.

Rathbun, M.J. 1907. South American Crustacea. *Revista Chilena de Historia Natural*, **11**: 45–50.

Ratnasingham, S. & Hebert, P.D. 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, **7**: 355–364.

Rhyne, A.L. & Anker, A. 2007. *Lysmata rafa*, n. sp., a new species of peppermint shrimp (Crustacea, Caridea, Hippolytidae) from the subtropical western Atlantic. *Helgoland Marine Research*, **61**: 291–296.

Rhyne, A.L. & Lin, J. 2006. A western Atlantic peppermint shrimp complex: re-description of *Lysmata wurdemanni*, description of four new species, and remarks on *Lysmata rathbunae* (Crustacea: Decapoda: Hippolytidae). *Bulletin of Marine Science*, **79**: 165–204.

Rhyne, A.L., Calado, R. & Dos Santos, A. 2012. *Lysmata jundalini*, a new peppermint shrimp (Decapoda, Caridea, Hippolytidae) from the Western Atlantic. *Zootaxa*, **3579**: 71–79.

Rhyne, A.L., Lin, J. & Deal, K.J. 2004. Biological control of aquarium pest anemone *Aiptasia pallida* Verrill by peppermint shrimp *Lysmata* Risso. *Journal of Shellfish Research*, **23**: 227–229.

Risso, A. 1816. *Histoire Naturelle des Crustacés des Environs de Nice*. Librairie Grecque-Latine-Allemande, Paris.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D. & Hines, A.H. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist*, **37**: 621–632.

Samuel, V.K.D., Sreeraj, C.R., Krishnan, P., Parthiban, C., Sekar, V., Chamundeeswarfi, K., Immanuel, T., Shesdev, P., Purvaja, R. & Ramesh, R. 2016. An updated checklist of shrimps on the Indian coast. *Journal of Threatened Taxa*, **8**: 8977–8988.

Sars, G.O. 1907. Mysidae. *Trudy Kaspiiskoi Ekspeditsii*, **1**: 243–313.

Soledade, G.O., Baeza, J.A., Boehs, G., Simões, S.M., Santos, P.S., Da Costa, R.C. & Almeida, A.O. 2013. A precautionary tale when describing species in a world of invaders: morphology, coloration and genetics demonstrate that *Lysmata rauli* is not a new species endemic to Brazil but a junior synonym of the Indo-Pacific *L. vittata*. *Journal of Crustacean Biology*, **33**: 66–77.

Spence Bate, C. 1888. Report on the Crustacea Macrura collected by the Challenger during the years 1873-76. *Report on the Scientific Results of the Voyage of H.M.S. Challenger During the Years* 1873–76. *Zoology*, **24**(52): 1–150.

Stebbing, T.R.R. 1915. South African Crustacea. Part VIII of S.A. Crustacea, for the Marine Investigations in South Africa. *Annals of the South African Museum*, **15**: 57–103.

Stebbing, T.R.R. 1921. Some Crustacea from Natal. *Annals of the Durban Museum*, **3**: 12–26.

Stimpson, W. 1860. Prodromus descriptionis animalium evertebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem, a Republic Federata missa, Cadwaladore Ringgold et Johanne Rodgers Ducibus, observavit et descripsit. Pars VIII, Crustacea Macrura. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **1860**: 22–47.

Stimpson, W. 1866. Descriptions of new Genera and Species of Macrurous Crustacea from the Coasts of North America. *Proceedings of the Chicago Academy of Sciences*, **1**: 46–48.

Thallwitz, J. 1891. Decapoden-Studien, insbesondere basirt auf A.B. Meyer's Sammlungen im Ostindischen Archipel, nebst einer Aufzählung der Decapoden und Stomatopoden des Dresdener Museums. *Abhandlungen und Berichte des Königlichen Zoologischen und Anthropologisch-Etnographischen Museums zu Dresden*, **3**(3): 1–56.

Trifinopoulos, J., Nguyen, L-T., von Haeseler, A. & Quang Minh, B. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, **44**: W232–W235.

Tuckey, T.D. & Fabrizio, M.C. 2013. Influence of survey design on fish assemblages: implications from a study in Chesapeake Bay tributaries. *Transactions of the American Fisheries Society*, **142**: 957–973.

d'Udekem d'Acoz, C. 2000. Redescription of *Lysmata intermedia* (Kingsley, 1879) based on topotypical specimens, with remarks on *Lysmata seticaudata* (Risso, 1816) (Decapoda, Caridea, Hippolytidae). *Crustaceana*, **73**: 719–735.

Vaughan, D.B., Grutter, A.S., Costello, M.J. & Hutson, K.S. 2016. Cleaner fishes and shrimp diversity and a re-evaluation of cleaning symbioses. *Fish and Fisheries*, **18**: 698–716.

Vaughan, D.B., Grutter, A.S. & Hutson, K.S. 2018a. Cleaner shrimp are a sustainable option to treat parasitic disease in farmed fish. *Scientific Reports*, **8**: 13959 [https://doi.org/10.1038/s41598-018-32293-6].

Vaughan, D.B., Grutter, A.S. & Hutson, K.S. 2018b. Cleaner shrimp remove parasite eggs on fish cages. *Aquaculture Environment Interactions*, **10**: 429–436.

Walsh, M.G., Lantry, B.F., Boscarino, B., Bowen, K., Gerlofsma, J., Schaner, T., Back, R., Questel, J., Smythe, A.G., Cap, R., Goehle, M., Young, B., Chalupnicki, M., Johnson, J.H. & McKenna, J.E. 2010. Early observations on an emerging Great Lakes invader *Hemimysis anomala* in Lake Ontario, *Journal of Great Lakes Research*, **36**: 499–504.

Wang, Y.-R. & Sha, Z.-L. 2018. Description of two new species of *Lysmata* Risso, 1816 (Decapoda, Lysmatidae) from the China seas, with remarks on *Lysmata vittata* (Stimpson 1860). *Zootaxa*, **4392**: 28–40.

Wicksten, M.K. 2000. The species of *Lysmata* (Caridea: Hippolytidae) from the Eastern Pacific Ocean. *Amphipacifica*, **2**: 3–22.

Wicksten, M.K. 2009. Interactions with fishes of five species of *Lysmata* (Decapoda, Caridea, Lysmatidae). *Crustaceana*, **82**: 1213–1223.

WoRMS. 2021a. *Lysmata* Risso, 1816. [http://www.marinespecies.org/aphia.php?p=taxdetails&id=106992] (accessed 3 December 2021).

WoRMS. 2021b. *Lysmata vittata* (Stimpson, 1860). [http://www.marinespecies.org/aphia.php?p=taxdetails&id=220261] (accessed 2 March 2021).

Yaldwyn, J.C. 1971. Preliminary descriptions of a new genus and twelve new species of natant decapod Crustacea from New Zealand. *Records of the Dominion Museum*, **7**: 85–94.

Yang, H. & Kim, C.H. 2010. Zoeal stages of *Lysmata vittata* (Decapoda: Caridea: Hippolytidae) reared in the laboratory. *Animal Systematics, Evolution and Diversity*, **26**: 261–278.

Zhan, A., Macisaac, H.J. & Cristescu, E.E. 2010. Invasion genetics of the *Ciona intestinalis* species complex: from regional endemism to global homogeneity. *Molecular Ecology*, **19**: 4678–4694.