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 (Kumshick 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. terramentis De Man, 1902, and L. tristisca 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, L. vitata 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’Udeken d’Aczo, 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., uniramous, 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. deletae Bruce, 1983, L. grahami Gordon, 1935, and L. splendidula 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. vitata, L. wurdemannii 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. baika Rhyne & Lin, 2006, L. californica, L. olavi Fransen, 1991, L. nayarensis 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 uniramous accessory branch (e.g., L. baika Baeza & Anker, 2008), L. vitata (the type species for Hippolysmata), as well as combinations of the genera Exhippolysmata Stebbing, 1915, Lysmatalle 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 arvedensis Giraldes, Macedo, Brandão, Baeza & Freire, 2018 (considered a junior synonym of Lysmata unicorns Holthuis & Maurin, 1952 by González-Ortegón et al., 2020), which possesses an uniramous 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. vitata, described from material collected in Hong Kong during the North Pacific Exploring Expedition (1853–1856; Stimpson, 1860). Lysmata vitata 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; Kensey, 1981; Bruce, 1990; Chace, 1997; Martín et al., 2012; De Grave et al., 2014; Anker & De Grave, 2016; Samuel et al., 2016). Lysmata vitata is also reported as introduced to Brazil (Soledade et al., 2013), Caribbean Panama (Pachelle et al., 2018) and Mediterranean Egypt (Abdeslam, 2018), and despite its widespread Indo-West Pacific distribution, as potentially cryptogenic in New Zealand (Ahyong, 2010).

Several species are considered to be synonyms of L. vitata, including Nauticaris unirecens Spencer Bate, 1860 (type locality: Hong Kong), Hippolysmata vitata var. subulata Hallwitz, 1891 (type locality: Philippines), Hippolysmata durbanensis Stebbing, 1921 (type locality: South Africa), Lysmata rauli Laubenheimer & Rhine, 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 & Rhine, 2010). The authors determined it was a novel species based on a
UNRESOLVED TAXONOMY CONFOUNDS THE LYSMATA VITTATA SPECIES COMPLEX

Table 1. Dorsal antennule and accessory branch structure of Lysmata species lacking a well-developed accessory branch. *Lysmata arocordensis is regarded as a junior synonym of L. unicorinis 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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Antennule structure</th>
<th>Relevant citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysmata amboinensis (De Man, 1888)</td>
<td>uniramous</td>
<td>Chace, 1997</td>
</tr>
<tr>
<td>Lysmata anchisteus Chace, 1972</td>
<td>vestigial</td>
<td>Chace, 1972</td>
</tr>
<tr>
<td>Lysmata ankeri Rhyne &amp; Lin, 2006</td>
<td>one segment vestigial</td>
<td>Fiedler et al., 2010</td>
</tr>
<tr>
<td>Lysmata arvoredensis Giraldes, Macedo, Brandão, Baeza &amp; Freire, 2018</td>
<td>one segment vestigial</td>
<td>Pachelle et al., 2020</td>
</tr>
<tr>
<td>Lysmata bahia Rhyne &amp; Lin, 2006</td>
<td>long accessory ramus</td>
<td>Pachelle et al., 2020</td>
</tr>
<tr>
<td>Lysmata bauern Prakash &amp; Baeza, 2017</td>
<td>free distal segment</td>
<td>Prakash &amp; Baeza, 2017</td>
</tr>
<tr>
<td>Lysmata boggesi Rhyne &amp; Lin, 2006</td>
<td>[not reported]</td>
<td>Rhyne &amp; Lin, 2006*</td>
</tr>
<tr>
<td>Lysmata brevirostrus Wang &amp; Sha, 2018</td>
<td>one segment vestigial</td>
<td>Wang &amp; Sha, 2018</td>
</tr>
<tr>
<td>Lysmata californica (Stimpson, 1866)</td>
<td>fused or with 1 free segment</td>
<td>Wicksten, 2000</td>
</tr>
<tr>
<td>Lysmata debelius Bruce, 1983</td>
<td>uniramous</td>
<td>Bruce, 1983</td>
</tr>
<tr>
<td>Lysmata dispar Hayashi, 2007</td>
<td>one segmented</td>
<td>Hayashi, 2007</td>
</tr>
<tr>
<td>Lysmata grabhami (Gordon, 1935)</td>
<td>single free segment</td>
<td>Pachelle et al., 2020</td>
</tr>
<tr>
<td>Lysmata gracillostris Wicksten, 2000</td>
<td>fused with 1–2 free segments</td>
<td>Wicksten, 2000</td>
</tr>
<tr>
<td>Lysmata guamensis Anker &amp; Cox, 2011</td>
<td>stump like</td>
<td>Anker &amp; Cox, 2011</td>
</tr>
<tr>
<td>Lysmata hochi Baeza &amp; Anker, 2008</td>
<td>unguis-shaped</td>
<td>Baeza &amp; Anker, 2008, Jose et al., 2020</td>
</tr>
<tr>
<td>Lysmata kemp Chace, 1997</td>
<td>uniramous</td>
<td>Froglia &amp; Deval, 2013</td>
</tr>
<tr>
<td>Lysmata kuekenthali (De Man, 1902)</td>
<td>unguis-shaped</td>
<td>Baeza and Anker, 2008</td>
</tr>
<tr>
<td>Lysmata lepotaclxytus Gan &amp; Li, 2016</td>
<td>one-segmented finger-like</td>
<td>Ashrafi et al., 2021</td>
</tr>
<tr>
<td>Lysmata lipkei Okuno &amp; Fiedler, 2010</td>
<td>one-segmented vestigial</td>
<td>Gan &amp; Li, 2016</td>
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<tr>
<td>Lysmata lepotaclxytus Gan &amp; Li, 2016</td>
<td>single free segment</td>
<td>Pachelle et al., 2016</td>
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<td>single free segment</td>
<td>Ashrafi et al., 2021</td>
</tr>
<tr>
<td>Lysmata morelandi (Yaldwyn, 1971)</td>
<td>one-segmented</td>
<td>Yaldwyn, 1971</td>
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<tr>
<td>Lysmata multiscissa (Nobili, 1904)</td>
<td>one-segmented vestigial</td>
<td>Hayashi, 2007</td>
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<tr>
<td>Lysmata nayardensis Wicksten, 2000</td>
<td>fused or with 1–2 free segments</td>
<td>Wicksten, 2000</td>
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<tr>
<td>Lysmata olavoi Fransen, 1991</td>
<td>fused</td>
<td>Fransen, 1991</td>
</tr>
<tr>
<td>Lysmata parvispinosus Wang &amp; Sha, 2018</td>
<td>one-segmented vestigial</td>
<td>Wang &amp; Sha, 2018</td>
</tr>
<tr>
<td>Lysmata pedersonii Rhyne &amp; Lin, 2006</td>
<td>[not reported]</td>
<td>Rhyne &amp; Lin, 2006*</td>
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<tr>
<td>Lysmata philippinensis Chace, 1997</td>
<td>one-segmented vestigial</td>
<td>Chace, 1997</td>
</tr>
<tr>
<td>Lysmata porti (Rathbun, 1907)</td>
<td>one-segmented finger-like</td>
<td>Rathbun, 1907</td>
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<tr>
<td>Lysmata rafa Rhyne &amp; Anker, 2007</td>
<td>rudimentary</td>
<td>Rhyne &amp; Anker, 2007</td>
</tr>
<tr>
<td>Lysmata rathbunae Chace, 1970</td>
<td>rudimentary</td>
<td>Chace, 1972</td>
</tr>
<tr>
<td>Lysmata rau Laubenuerhimer &amp; Rhyne, 2010</td>
<td>uniramous</td>
<td>Soledade et al., 2013*</td>
</tr>
<tr>
<td>Lysmata splendida Burukovsky, 2000</td>
<td>uniramous</td>
<td>Burukovsky, 2000</td>
</tr>
<tr>
<td>Lysmata stenolepis Crosnier &amp; Forest, 1973</td>
<td>small elongated lobe with 3 articulated bristles</td>
<td>Crosnier &amp; Forest, 1973</td>
</tr>
<tr>
<td>Lysmata udoi Baeza, Bolaños, Hernandez &amp; López, 2009</td>
<td>rudimentary</td>
<td>Baeza et al., 2009a</td>
</tr>
<tr>
<td>Lysmata uncinornis Holthuis &amp; Maurin, 1952</td>
<td>hook-shaped</td>
<td>Holthuis &amp; Maurin, 1952</td>
</tr>
<tr>
<td>Lysmata vittata (Stimpson, 1860)</td>
<td>uniramous</td>
<td>González-Ortegón et al., 2020</td>
</tr>
<tr>
<td>Lysmata wurdemannii (Gibbes, 1850)</td>
<td>two-segmented</td>
<td>Stimpson, 1860; Bruce, 1990;</td>
</tr>
<tr>
<td></td>
<td>rudimentary secondary ramus</td>
<td>Marin et al., 2012</td>
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<td></td>
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<td>Kubo, 1951</td>
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</tbody>
</table>

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 unguiniform 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. vallii* from Brazil in Laubenheimer & Rhyne (2014), 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 (Abdelsalam, 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 lymnatine shrimps. Ancodetal data suggest *L. vittata* that possess transverse bands as described above and/or unguiniform 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 unguiniform accessory branch, 20 (Bruce, 1990), 19–22 (Hayashi & Miyake, 1968), and 18–21 (Ahyong, 2010). Limited data also suggest there may be differences in zonal 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, 2017). The type material for *N. unicaudatus* 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 spine on the distal article of the antennular peduncle, and articulation of the ischiium 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* Gibbs, 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 vouched 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, unprocessed 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; Ratmasingham & Hebert, 2007). All North American DNA 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 material, and 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 included. DNA was extracted using the Autogen Prep 965 phenol-chloroform automated extractor (Autogen, Holliston, MA, USA).

**DNA amplification and sequencing**

For fresh North American samples, we amplified a ~500 bp fragment of the 16S rRNA gene using the universal primers 16Sar (GCGCTGTATATC AAA A CAT) and 16Sbr (CCGGTCTGAATCGAGTACGT) of Palumbi et al. (1991). The 16Sar/16Sbr primer set performed poorly with the 20-30 year-old ethanol preserved museum material. To increase sequencing success, we designed *Lysmata*-specific primers, 16SF_Lysmata (GCCGTYAHHTGACCGTGCA AAGG) and 16SR_Lysmata (TTAATTCAAC ATCGAGTCCG), 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 buffer (Bioline, Cincinnati, OH, USA), 0.3 µM each primer, 2.5 mM MgCl₂, 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 Sequencer) 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, Schenzhen, 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 (*Exshipolysmata eniostris* Kemp 1914, *Ligur hispifera* Risso, 1816, *Lysmatella prima* Borradaille, 1915, and *Momincas heterocarpoida* Nobili, 1903), and three sequences of non-lysmatid shrimps (*Merguiola oligodon* De Man, 1888, *Mergui rhizophorae* Rathbun, 1900, and *Taenzia carolinensis* 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 “_” 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 seedling 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. raui 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 (Martinez-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 lymmatid 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. raui (Laubenheimer & Ryhne, 2010) and subsequent analysis of additional Brazilian material of L. raui re-classified as L. vittata (Soledade et al., 2013; Alves et al., 2018), as well as L. vittata from Egypt (Abdelsalam, 2018), there were clear and consistent morphological differences between the North American L. vittata and L. raui/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. raui/ vittata from Brazil (Laubenheimer & Ryhne, 2010; Soledade et al., 2013; Alves et al., 2018, 2019; Mediterraneana (Abdelsalam, 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. ambdouisensis 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 separate species. Five individuals from four separate lots (NTM: Cr004808, Cr004904, Cr009751, and Cr009787) were most similar to the original description of L. raui and L. vittata from Brazil and Egypt, with some minor differences, and were identified as L. cf. raui. 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 ischiial articles of the second pereopod as barely visible in L. lipkei, whereas the same articles are distinct in L. dispar (Hayashi, 2007). The ischiial articles were clearly noticeable in the present material, albeit less distinct than the meral and carpal articles. The rostrum was straight (similar
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).

**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.
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. gracilis* (Woestken, 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. california* and *L. olavii* 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. ambioensis, 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.

We re-examined five of the seven specimens of *L. vittata* (all but NIWA [MIT5] 13209) collected in northern New Zealand (Auckland and Kaipara Harbor) previously reported in Ahlyong (2010), plus an additional *L. vittata* collected from Whangārei, New Zealand (NIMA [MIT5] 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 & Rhine (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
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 31341) with a carpal second pereopod count of 22–23 (and a meral count of 12–13), considerably higher than has previously been reported.

<table>
<thead>
<tr>
<th>Character</th>
<th>Lysmata vittata – Hong Kong (Stimpson, 1860; Bruce, 1990; Chace, 1997)</th>
<th>Lysmata vittata – USA (present study; N = 80)</th>
<th>Lysmata vittata – New Zealand (present study; N = 6; Ahyong, 2010)</th>
<th>Lysmata vittata – Brazil (Laubenheimer &amp; Rhyne, 2010; Soledade <em>et al.</em>, 2013; Pachelle <em>et al.</em>, 2020)</th>
<th>Lysmata cf. rauli – Australia (present study; N = 6)</th>
<th>Lysmata wurdemanni (Rhyne <em>et al.</em>, 2006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dorsal rostral teeth</td>
<td>6</td>
<td>6–8</td>
<td>7</td>
<td>6–8</td>
<td>8</td>
<td>4–6</td>
</tr>
<tr>
<td>Number of ventral rostral teeth</td>
<td>3</td>
<td>2–6</td>
<td>2–4</td>
<td>2–5</td>
<td>2–6</td>
<td>2–6</td>
</tr>
<tr>
<td>Rostrum length</td>
<td>Reaching distal margin of 2nd article of the antennular peduncle</td>
<td>Reaching distal margin of 2nd article of the antennular peduncle</td>
<td>Reaching distal margin of 2nd article of the antennular peduncle</td>
<td>Reaching midpoint of 2nd article of antennular peduncle</td>
<td>Reaching distal margin of 2nd article of the antennular peduncle</td>
<td>Reaching distal margin of 2nd article of the antennular peduncle</td>
</tr>
<tr>
<td>Pterygostomial tooth</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Number of articles on the accessory branch of the dorsal antennular flagellum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Stylodermite length</td>
<td>Short, reaching mid length of 1st article</td>
<td>Short, reaching mid length of 1st article</td>
<td>Short, reaching mid length of 1st article</td>
<td>Short, reaching mid length of 1st article</td>
<td>Short, reaching mid length of 1st article</td>
<td>Short, not reaching distal margin of 1st article</td>
</tr>
<tr>
<td>Antennal scale (scaphocerite)</td>
<td>Overreaching antennular peduncle, 3x long as wide</td>
<td>Overreaching antennular peduncle, 3x long as wide</td>
<td>Overreaching antennular peduncle, 3x long as wide</td>
<td>Overreaching antennular peduncle, 3x long as wide</td>
<td>Overreaching antennular peduncle, 3x long as wide</td>
<td>Distinctly overreaching antennular peduncle, 4x long as wide</td>
</tr>
<tr>
<td>Number of second pereopod carpal articles</td>
<td>15–31</td>
<td>18–23</td>
<td>19–21</td>
<td>15–19 (22–23)*</td>
<td>17–26</td>
<td>30–33</td>
</tr>
<tr>
<td>Coloration</td>
<td>Numerous fine red stripes</td>
<td>Thin red stripes alternating in pigment density, without transverse bands</td>
<td>Undetermined</td>
<td>Thin red stripes, with transverse red bands at 1st and between 3rd and 4th somites</td>
<td>Undetermined</td>
<td>Thin red longitudinal stripes with oblique banding ventrally</td>
</tr>
</tbody>
</table>
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 (Bayesian 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 *Mergui* species (posterior probability; *p* = 100; ML = 100) was sister to the clade comprising all *Eucheliphenoma, Lysmatella*, *Lysmatina* and *Mmeei*, though with moderate to low support (*p* = 63; ML < e50). 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* = 97; ML = 98), which was sister to *L. prima* (*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 0.106–0.204 (Supplementary material Table S3). The remaining groups, broadly called “Rauli Type”, comprised two sister clades (*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. argentaupunctata/L. tetratensis* (0.082), *L. galapagensis/L. moorei* (0.86), *L. ankeri/L. pedersenii* (0.09), *L. boggessi/L. rafa* (0.093), *L. intermedia/L. nilita* (1.04), and *L. graciliorstris/L. wurdemannii* (0.0108), as well as between *M. oligodon* and *M. rhizophore* (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* = 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. dispers* 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* = 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 uniramous accessory branch, except for *L. arvordenisi* (*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. ensiorstris, L. prima*, and *M. heterocaptoides*, were recovered in a strongly supported clade (*p* = 100; ML = 98), rendering *Lysmata* paraphyletic. This first monophyletic clade was analogous to the “Morpho-variable” clade of Baeca (2010; Fig. 4). The second highly supported monophyletic clade (*p* = 100; ML = 97) consisted of all species of *Lysmata* possessing a multi-article accessory branch (*L. argentaupunctata, L. galapagensis, L. intermedia, L. moorei, L. nilita, L. seticaudata, L. tetratensis, and L. cf. tetratensis*) and was analogous to the “Long Branch” clade of Fiedler et al. (2010) and the “Cosmopolitan” clade of Baeca (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 Rhyme & Lin, 2006, L. boggessi Rhyme & Lin, 2006, L. bahia, L. debelius, L. grabhami, L. pedersenii Rhyme & Lin, 2006, L. rafa Rhyme & Anker, 2007, and L. wurdemannii*), the species described as possessing a variable dorsal antennule (i.e., uniramous or with 1 or 2 articles; *L. californica, L. graciliorstris, and L. nagiarenitensis*) and *L. arvordenisi*, which possesses an uniramous accessory branch formed a poorly supported clade (*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. olavo* and placement of *L. vittata* sensu Bruce (1990) in the “Morpho-variable” clade of Baeca (2010; Fig 4).

There was also little support for a “Specialized Cleaner Shrimp” clade of Fiedler et al. (2010) and “Cleaner” clade of Baeca (2010), as *L. amboinensis, L. debelius, and L. grabhami* were recovered in a poorly supported clade (*p* = 53; ML < 50). The third strongly supported clade consisted of species in the greater *L. wurdemannii* species complex (L. ankeri, L. boggessi, L. pedersenii, L. rafa, and L. wurdemannii) from the Caribbean/Gulf of Mexico/western Atlantic and *L. graciliorstris* from the eastern Pacific, which was analogous to the “Tropical American” clade of Baeca (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. ensiorstris* (*p*-distance  = 0.122), and *M. heterocaptoides* (*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 *Mergui*. 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. vitatta*/*L. rauli* from Brazil (15–19; Laubenheimer & Rhyne, 2010; Soledade 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. vitatta* 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. vitatta* possessed numerous thin red stripes without transverse bars, similar to *L. vitatta* 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. vitatta* collected in Brazil (Alves et al., 2018, 2019), as well as *L. vitatta* reported from Australia (Barton et al., 2020), India (Kemp, 1914), Caribbean Panama (Pachelle et al., 2018), Singapore (Anker & De Grave, 2016), and Egypt (Abdelsalam, 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. vitatta* and the toptotypic 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. “vitatta”* 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. vitatta* 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. vitatta*” and warrants reexamination to confirm identification. The rostrum of the same North American and New Zealand *L. vitatta* and the toptotypic 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. “vitatta” 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. “vitatta” 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. vitatta*. 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 Zoaa I and the number of aesthetasc and setae on the outer flagellum of the antennule of the Zoaa III between individuals collected in India and Korea/southeastern Russia (Pillai, 1966; Yang & Kim, 2010; Marin et al., 2012). Adult *L. vitatta* from southeastern Russia collected concurrently to these zoas 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. vitatta* 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 interspecífic differences among the constituent entities rather than an extraordinarily wide intraspecific variation. Historically, *L. vitatta* 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. vitatta* 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. vitatta* 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 chela 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. vitatta* sensu Bruce (1990), *L. rauli*, or other *Lysmata* species.

The existence of a *L. vitatta* species complex and the taxonomic importance of coloration patterns in delimiting *Lysmata* species are highlighted by the small number of *L. vitatta* 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. vitatta* 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. vitatta*, 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 reevaluating 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. arsorensis/L. uncinorius* 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 *Lysmatidae* 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. vitatta* 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 Hippolyssma 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. olavoii. Although the apparent homoplasy of a non-multi-article accessory branch certainly undermines the recognition of both Lysmata and Hippolyssma 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 Hippolyssma would not be available to any of the species once included in Hippolyssma, 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 L. anchisteus, L. hochi, L. lipkei, L. prama, M. uncicornis, 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. dispers, 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. kuenkelti De Man, 1902, L. morelandi, L. multisicca, L. philippinensis Chace, 1997, L. stenolepis Cronin & 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/

**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 Hippolyssma (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 Gr04983) 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. dispers/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 of the junior synonyms remains uncertain until fully revised, since, as noted above, the type material of N. unirecessus is in poor condition and the type material for H. vittata var. subtiliss 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. darwini*is (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 antennal 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 & Ryhne, 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. (2010) 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 (Ablesalam, 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. ambienensis*). 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**

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 (Baeva, 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. wurdemanni* 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 *Hemiopsyans anoma Lars*, 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 (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 lyomatid 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.*; Ryhne et al., 2004) and *Aeropore*-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 (Baeva & 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 subtemperate 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 (Bacea, 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 simultaneous hermaphroditism (PSH). In PSH, young individuals first mature as males and later transition to a phenotypically female simultaneous hermaphroditism (PSH). In PSH, young individuals first mature as males and later transition to a phenotypically female simultaneous hermaphroditism (PSH). In PSH, young individuals first mature as males and later transition to a phenotypically female simultaneous hermaphroditism (PSH).

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 33630133) and Waikato (observation 33997880) in September, 2019 and western Auckland (observation 73551963) and eastern Auckland (observation 73551365) in March, 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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