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Rediscovery of *Sagittalarva inornata* n. gen., n. comb. (Gilbert, 1890) (Perciformes: Labridae), a long-lost deepwater fish from the eastern Pacific Ocean: a case study of a forensic approach to taxonomy using DNA barcoding

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Abstract

Some of the more valuable contributions of a standardized DNA sequence database (the DNA barcode) are matching specimens of different life stages and confirming the species identity of individuals from distant locations. These applications can facilitate the detective work required to solve difficult taxonomic problems. In this case, a match was made between the COI mtDNA sequence of an adult male wrasse recently caught at the tip of Baja California in Mexico in deep water (30–100m) and sequences from a series of unusual larvae collected about 3500 km to the south, in the open ocean over the Galápagos Rift hydrothermal vents in 1985. The Baja adults fit the recent description of *Halichoeres raisneri* Baldwin & McCosker, 2001 from the Galápagos and Cocos Islands. However, another deepwater labrid is known from the same site and depth in Baja; it is the type locality for the century-old holotype and only specimen of the Cape Wrasse *Pseudojulis inornatus* Gilbert, 1890 (later as *Pseudojuloides inornatus*). Deepwater video images from the tip of Baja show wrasses identical to *H. raisneri* photographed in Galápagos but who also fit the description of *Pseudojulis inornatus*. This coincidence led to a closer investigation of the holotype with x-ray, which revealed unanticipated caniniform teeth (vs. incisiform in *Pseudojuloides*) and an error in the fin-ray count in the original description, both of which mistakenly separated *Halichoeres raisneri*. The two species now match in markings, meristics, and morphology as well as overlapping range and are therefore synonymized. Phenetic and phylogenetic trees using mtDNA and nuclear DNA sequences show the species is not close to any other lineage and does not group with the other julidine labrids of the New World or the *Pseudojuloides* or *Halichoeres* of the Indo-Pacific. The distinctive larval morphology, long, thin, and flattened with a sharply pointed black-tipped snout, resembles no other described labrid larvae and, without an available genus, the new genus *Sagittalarva* Victor, n. gen. and the new combination *Sagittalarva inornata* (Gilbert, 1890), n. gen., n. comb. are described.

Key words: phylogenetics, taxonomy, DNA, barcoding, labrid, *Halichoeres*, *Pseudojuloides*, *Sagittalarva*, Galápagos, Eastern Pacific, deep, larvae, wrasses, forensics

Introduction

The advent of highly efficient DNA sequencing and the selection of a standardized mtDNA segment for identifying taxa has resulted in the Barcode of Life database (BOLD) that holds great promise for solving biogeographic and taxonomic questions (Hebert *et al.* 2003; Meier 2008; Ward *et al.* 2009). The convoluted history of the Cape Wrasse provides a clear example of how DNA sequences can help resolve complex taxonomic problems and how a forensic-type approach can reveal unexpected connections and clarify the status of little known taxa. Shared DNA sequences identify and unite different life stages, including larvae and the often different juvenile, immature, female, and male color forms characteristic of many reef fishes. Furthermore, with the addition of other mitochondrial and nuclear genes, DNA sequences can provide a powerful tool for resolving genetic and evolutionary relationships. These phylogenetic analyses are particularly useful in species-rich genera with few distinguishing morphological characters, such as those of the labrid fishes (Bernardi *et al.* 2000; 2004; Rocha 2004; Rocha *et al.* 2005, Barber & Bellwood 2005; Westneat & Alfaro 2005).

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Until recently, the taxonomy of many shorefishes living in deep water has suffered from scant collections and uncertain ranges. The introduction of mixed-gas deep diving and submersibles has alleviated the problem to a degree and led to the recent discovery of new deepwater fish species in many locations (e.g. Pyle *et al.* 2008; McCosker & Rosenblatt 2010). Nevertheless, the majority of these fishes are known from few individuals and restricted locations. The eastern Pacific shorefish fauna is no exception and includes a number of poorly-known species in this category. In this study, we document the odyssey of the Cape Wrasse, originally described as *Pseudojulis inornatus* Gilbert, 1890, from its discovery more than a century ago and its unrecognized reemergence, as larvae, females, and finally males, in various collections under various names over the decades.

The julidine labrids comprise a large group of about 200 species of mostly reef-associated wrasses found in all tropical and subtropical seas (Parenti & Randall 2000, Kuiter 2010). The genus *Halichoeres* is the largest genus within the group, with about 80 species at the most recent count (Parenti & Randall 2011). Phylogenetic studies indicate that *Halichoeres* is a troublesome polyphyletic collection of lineages without good morphological characters to either unify them or separate them into distinct clades (Barber & Bellwood 2005, Westneat & Alfaro 2005). Fortunately, the julidine labrids in the New World are more easily categorized and almost all of the species fall into two large monophyletic groupings; one broad clade of *Halichoeres* with about 20 species (including *Oxyjulis californica* (Günther)) and one of *Thalassoma* with 7 species (Bernardi *et al.* 2004; Rocha 2004; Barber & Bellwood 2005; Luiz *et al.* 2009; Rocha *et al.* 2010; this study). The exceptions are the Caribbean *Halichoeres maculipinna* (Müller & Troschel), with its Brazilian sibling *H. penrosei* Starks, who are markedly divergent and lie closer to *Thalassoma* than *Halichoeres* in phylogenetic trees (Barber & Bellwood 2005). The other exception comprises the eastern Pacific *Halichoeres melanotis* (Gilbert, 1890), with its Cocos Island sibling *H. salmofasciatus* Allen & Robertson, 2002, who are also somewhat distinct from their New World congeners (Rocha *et al.* 2010; this study).

Several eastern Pacific species of *Halichoeres* have been historically separated into *Pseudojulis* based on the absence of a posterior canine tooth at the corner of the jaw; however, this character is variably expressed (Randall 1978) and, furthermore, those species lie within different clades and among close relatives with posterior canines (Barber & Bellwood 2005; Rocha *et al.* 2010; this study). *Pseudojulis* is an invalid genus and those species have mostly been returned to *Halichoeres* (Randall 1978; Parenti & Randall 2000).

The Cape Wrasse was originally described as *Pseudojulis* based on the absence of the posterior canines (Gilbert 1890). More recently, it was assigned to the Indo-Pacific genus *Pseudojuloides* (Parenti & Randall 2000; Robertson & Allen 2008). However it shares only a fusiform shape with *Pseudojuloides* and has neither the characteristic chisel-like dentition nor the larval form of that genus and it does not group with them in phylogenetic trees. Without an available genus for the species and given its genetic distance from other julidine clades, along with its distinctive larval stage, a new monotypic genus is erected for the species. Since prior type specimens have all been juveniles or females, the colorful adult male form is described. Based on collections and underwater videos, the species ranges widely in the eastern Pacific Ocean, from the Sea of Cortez to Colombia and the offshore islands of Cocos, Galápagos, and Malpelo.

Material and methods

The fresh adult specimens from Baja California were caught with hook and line by John Snow off San Jose del Cabo in 2007 at 50–100m depth at Gordo Bank (23.02°N, 109.50°W) and San Luis Canyon (23.05°N, 109.40°W). The larval series (31 larvae) were collected in oblique plankton-net tows to about 100m in mile-deep waters over the mid-ocean Galápagos Rift hydrothermal vents during the March 1985 *Alvin* expedition to explore the Rose Garden vents (0.81°N, 86.23°W). The site is about 400 km E of the Galápagos Islands and 600 km W of the South American mainland (Victor 1987). Specimens were preserved in 90% ethanol and are deposited in the Marine Vertebrate Collection at Scripps Institution of Oceanography (SIO).

Specimens for julidine COI mtDNA sequence comparisons were collected over the last three decades from Galápagos, Panama, Cocos, Clipperton, Revillagidos, and Baja California in the eastern Pacific Ocean; from Panama and Noronha, Brazil in the western Atlantic Ocean; and from Bali, Palau, Okinawa, Rangiroa, Rarotonga, and Hawaii in the Indo-Pacific Ocean (Table 1). Additional sequences were from GenBank records, many from Barber and Bellwood (2005). Sequences for a number of julidine species were provided by Dirk Steinke of the

Biodiversity Institute of Ontario and Allan Connell's collections via the South African Institute for Aquatic Biodiversity; several by Bob Ward of CSIRO Marine and Atmospheric Research, Hobart, Australia; sequences of *Halichoeres burekae* Weaver & Rocha and *Halichoeres cyanocephalus* (Bloch) were provided by Lourdes Vásquez Yeomans and Martha Valdez of ECOSUR in Chetumal, Quintana Roo, Mexico; *Halichoeres bathyphilus* (Beebe & Tee-Van) by Andrew Bentley of the Ichthyology Division, Biodiversity Institute, University of Kansas; and *Halichoeres melanochir* Fowler & Bean and *Thalassoma cupido* (Temminck and Schlegel) by Kwang-Tsao Shao of the Biodiversity Research Center, Academia Sinica, Taiwan. Specimens of *Pseudojuloides severnsi* Bellwood & Randall were obtained from the aquarium-fish trade. The sequences used to generate the maximum-likelihood phylogenetic tree in Fig. 11 are those listed in Westneat and Alfaro (2005), with the addition of the recent sequences of *Sagittalarva inornata* (GenBank accession numbers for the latter are JX684103–6 for Rag2, Tmo4c4, 12s, and 16s).

TABLE 1. GenBank accession numbers and collection data for the labrid species listed in the tree in Fig. 10. Sources from literature as follows: B & B 2005 (Barber & Bellwood 2005), Read 2006 (Read *et al.* 2006), and Hodge 2012 (Hodge *et al.* 2012).

Genus	species	GenBank number	Collection location	Collector/source
Anampses	*caeruleopunctatus*	JQ839396	Rarotonga	B. Victor
Anampses	*chrysocephalus*	JQ839398	Hawaii	B. Victor
Anampses	*geographicus*	DQ164156	Australia, GBR	Read 2006
Anampses	*lineatus*	FJ582831	Sri Lanka	D. Yanke
Anampses	*melanurus*	FJ582832	Philippines	D. Yanke
Anampses	*meleagrides*	DQ164157	Micronesia	Read 2006
Anampses	*neoguinaicus*	FJ582843	Tonga	D. Yanke
Anampses	*twistii*	JQ839401	Rangiroa	B. Victor
Coris	*atlantica*	GQ341590	Bay of Biscay	R. Hanel
Coris	*auricularis*	JQ839414	W. Australia	P. Last
Coris	*aurilineata*	AY850777	Australia	B & B 2005
Coris	*aygula*	JQ839415	Rarotonga	B. Victor
Coris	*batuensis*	AY850793	Australia, GBR	B & B 2005
Coris	*bulbifrons*	EF609338	S. Australia	G. Yearsly
Coris	*caudimacula*	JF493269	Mozambique	A. Connell
Coris	*cuvieri*	JQ839416	Bali	B. Victor
Coris	*dorsomacula*	AY850768	Australia, GBR	B & B 2005
Coris	*flavovittata*	AY850770	Hawaii	B & B 2005
Coris	*formosa*	HQ561485	Mozambique	A. Connell
Coris	*gaimard*	JQ839418	Bali	B. Victor
Coris	*julis*	GQ341591	Corsica	R. Hanel
Coris	*picta*	AY850767	Australia	B & B 2005
Coris	*pictoides*	AY850778	Bali	B & B 2005
Gomphosus	*caeruleus*	JF493550	Mozambique	A. Connell
Gomphosus	*varius*	JQ431774	French Polynesia	S. Planes
Halichoeres	*adustus*	JQ839442	Cocos Island	G. M. Wellington
Halichoeres	*argus*	JQ839444	Palau	B. Victor
Halichoeres	*bathyphilus*	JQ839445	Texas	K. McNyset
Halichoeres	*binotopsis*	AY850783	Indonesia	B & B 2005

......*continued on the next page*

TABLE 1. (Continued)

Genus	species	GenBank number	Collection location	Collector/source
Halichoeres	*biocellatus*	JX438018	Bali	B. Victor
Halichoeres	*bivittatus*	JQ839446	Caribbean Panama	B. Victor
Halichoeres	*brownfieldi*	AY850798	W. Australia	B & B 2005
Halichoeres	*burekae*	JN313704	Yucatan, Mexico	L. Vásquez Yeomans
Halichoeres	*chierchiae*	JQ839456	Pacific Panama	B. Victor
Halichoeres	*chloropterus*	FJ583491	Indonesia	D. Yanke
Halichoeres	*chrysus*	JQ839460	Bali	B. Victor
Halichoeres	*claudia*	FJ583531	Tonga	D. Yanke
Halichoeres	*cosmetus*	JF493590	Mozambique	A. Connell
Halichoeres	*cyanocephalus*	HM389563	Yucatan, Mexico	L. Vásquez Yeomans
Halichoeres	*dimidiatus*	JQ839461	Noronha, Brazil	B. Victor
Halichoeres	*discolor*	JQ839463	Cocos Island	G. M. Wellington
Halichoeres	*dispilus*	JQ839467	Baja California	B. Victor
Halichoeres	*garnoti*	JQ839469	Caribbean Panama	B. Victor
Halichoeres	*hartzfeldii*	JQ839471	Bali	B. Victor
Halichoeres	*hortulanus*	JQ839472	Bali	B. Victor
Halichoeres	*insularis*	JQ839474	Revillagigedos	G. M. Wellington
Halichoeres	*iridis*	JF493597	South Africa	P. Heemstra
Halichoeres	*kallochroma*	FJ583512	Indonesia	D. Yanke
Halichoeres	*lapillus*	JF493598	South Africa	A. Connell
Halichoeres	*leucoxanthus*	FJ583506	Sri Lanka	D. Yanke
Halichoeres	*leucurus*	AY850786	Indonesia	B & B 2005
Halichoeres	*maculipinna*	JQ839479	Caribbean Panama	B. Victor
Halichoeres	*margaritaceus*	JQ839482	Rangiroa	B. Victor
Halichoeres	*marginatus*	JQ839486	Rangiroa	B. Victor
Halichoeres	*melanochir*	JX034556	Taiwan	K.T. Shao
Halichoeres	*melanotis*	JQ839487	Pacific Panama	B. Victor
Halichoeres	*melanurus*	JQ839492	Bali	B. Victor
Halichoeres	*melasmapomus*	AY850788	Indonesia	B & B 2005
Halichoeres	*miniatus*	AY850795	Australia, GBR	B & B 2005
Halichoeres	*nebulosus*	JQ839493	Bali	B. Victor
Halichoeres	*nicholsi*	JQ839497	Galápagos	B. Victor
Halichoeres	*nigrescens*	AY850792	Australia, GBR	B & B 2005
Halichoeres	*notospilus*	JQ839501	Galápagos	B. Victor
Halichoeres	*ornatissimus*	FJ583525	Hawaii	D. Yanke
Halichoeres	*papilionaceus*	AY850784	Indonesia	B & B 2005
Halichoeres	*pelicieri*	JF493603	Mozambique	A. Connell
Halichoeres	*pictus*	JQ839502	Caribbean Panama	B. Victor
Halichoeres	*podostigma*	JQ952619	Bali	B. Victor
Halichoeres	*poeyi*	JQ839506	Caribbean Panama	B. Victor
Halichoeres	*prosopeion*	JX438019	Bali	B. Victor
Halichoeres	*radiatus*	JQ839513	Caribbean Panama	B. Victor

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TABLE 1. (Continued)

Genus	species	GenBank number	Collection location	Collector/source
Halichoeres	*richmondi*	AY850787	Indonesia	B & B 2005
Halichoeres	*salmofasciatus*	JQ839527	Cocos Island	G. M. Wellington
Halichoeres	*scapularis*	JQ839530	Bali	B. Victor
Halichoeres	*semicinctus*	JQ839532	Baja California	B. Victor
Halichoeres	*socialis*	AY850752	Belize	B & B 2005
Halichoeres	*solorensis*	AY850779	Indonesia	B & B 2005
Halichoeres	*tenuispinus*	EU082205	Korea	D.J. Oh
Halichoeres	*trimaculatus*	JQ839534	Rangiroa	B. Victor
Halichoeres	*trispilus*	HQ561458	South Africa	A. Connell
Halichoeres	*vrolikii*	FJ583514	Indonesia	D. Yanke
Halichoeres	*zeylonicus*	FJ158564	India	W.S. Lakra
Halichoeres	*zulu*	JF493601	South Africa	A. Connell
Halichoeres	sp. aff. *chrysus*	FJ583532	Indo-Pacific	D. Yanke
Hemigymnus	*fasciatus*	JQ839536	Bali	B. Victor
Hemigymnus	*melapterus*	JX034557	Okinawa	B. Victor
Hologymnosus	*annulatus*	AY850772	Micronesia	B & B 2005
Hologymnosus	*doliatus*	JQ839537	Rarotonga	B. Victor
Labrichthys	*unilineatus*	AY850750	Australia, GBR	B & B 2005
Labroides	*bicolor*	JF435041	French Polynesia	S. Planes
Labroides	*dimidiatus*	JX034558	Bali	B. Victor
Labroides	*pectoralis*	JQ839545	Palau	B. Victor
Labroides	*phthirophagus*	DQ521031	Hawaii	K. Longenecker
Labropsis	*polynesica*	JF435045	French Polynesia	S. Planes
Leptojulis	*chrysotaenia?*	JQ839546	Bali	B. Victor
Leptojulis	*cyanopleura*	AY850747	Australia, GBR	B & B 2005
Macropharyngodon	*bipartitus*	JF493851	Mozambique	A. Connell
Macropharyngodon	*choati*	AY850774	Australia, GBR	B & B 2005
Macropharyngodon	*cyanoguttatus*	DQ164150	Mauritius	Read 2006
Macropharyngodon	*geoffroy*	JQ839547	Hawaii	B. Victor
Macropharyngodon	*kuiteri*	DQ164152	Australia, GBR	Read 2006
Macropharyngodon	*meleagris*	JX438020	Rarotonga	B. Victor
Macropharyngodon	*negrosensis*	JQ839548	Bali	B. Victor
Macropharyngodon	*ornatus*	JQ839550	Bali	B. Victor
Ophthalmolepis	*lineolata*	JQ839560	W. Australia	E. Lek
Oxyjulis	*californica*	GU440440	California	J. Hyde
Parajulis	*poecilepturus*	GU207334	Japan	M. Kato
Pseudocoris	*aurantiofasciata*	JF435148	French Polynesia	S. Planes
Pseudocoris	*bleekeri*	FJ583992	Vietnam	D. Yanke
Pseudocoris	*heteroptera*	AY850765	Australia, Coral Sea	B & B 2005
Pseudocoris	*yamashiroi*	JQ839565	Bali	B. Victor
Pseudocoris	sp. aff. *yamashiroi*	JQ839567	Bali	B. Victor
Pseudojuloides	*atavai*	JQ839568	Rarotonga	B. Victor

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TABLE 1. (Continued)

Genus	species	GenBank number	Collection location	Collector/source
Pseudojuloides	*cerasinus*	JQ839570	Hawaii	B. Victor
Pseudojuloides	*severnsi*	JQ839574	Indo-Pacific	Aquarium trade
Sagittalarva	*inornata*	JQ952620	Baja California	J. Snow
Stethojulis	*albovittata*	HQ561472	Mozambique	A. Connell
Stethojulis	*balteata*	JQ839596	Hawaii	B. Victor
Stethojulis	*bandanensis*	JQ839601	Rangiroa	B. Victor
Stethojulis	*interrupta*	JQ839603	Bali	B. Victor
Stethojulis	*strigiventer*	JQ839605	Okinawa	B. Victor
Stethojulis	*trilineata*	JQ839608	Okinawa	B. Victor
Suezichthys	*gracilis*	GU673132	NE Australia	D. Gledhill
Thalassoma	*amblycephalum*	JQ839613	Bali	B. Victor
Thalassoma	*ballieui*	DQ521017	Hawaii	K. Longenecker
Thalassoma	*bifasciatum*	JQ839615	Caribbean Panama	B. Victor
Thalassoma	*cupido*	JX034560	Taiwan	K.T. Shao
Thalassoma	*duperrey*	DQ443874	Hawaii	Aquarium trade
Thalassoma	*genivittatum*	GU805114	South Africa	A. Connell
Thalassoma	*grammaticum*	JQ839618	Clipperton	D. R. Robertson
Thalassoma	*hardwicke*	JX034561	Rarotonga	B. Victor
Thalassoma	*hebraicum*	JF494675	Mozambique	A. Connell
Thalassoma	*heiseri?*	JQ839611	Rarotonga	B. Victor
Thalassoma	*jansenii*	JQ952621	Bali	B. Victor
Thalassoma	*loxum?*	JQ952622	Sri Lanka	D. Yanke
Thalassoma	*lucasanum*	JQ839623	Galápagos	B. Victor
Thalassoma	*lunare*	JX438021	Bali	B. Victor
Thalassoma	*lutescens*	JQ839624	Rarotonga	B. Victor
Thalassoma	*nigrofasciatum*	DQ443870	Australia, Coral Sea	F. Walsh
Thalassoma	*noronhanum*	JQ839625	Noronha, Brazil	B. Victor
Thalassoma	*pavo*	GQ341611	Corsica	R. Hanel
Thalassoma	*purpureum*	JQ839635	Galápagos	B. Victor
Thalassoma	*quinquevittatum*	JX034564	Palau	B. Victor
Thalassoma	*robertsoni*	JQ839642	Clipperton	D. R. Robertson
Thalassoma	*trilobatum*	JQ839648	Hawaii	B. Victor
Thalassoma	*virens*	JQ839654	Baja California	B. Victor

DNA extractions were performed at the Biodiversity Institute of Ontario with the NucleoSpin96 (Machery-Nagel) kit according to manufacturer specifications under automation with a Biomek NX liquid-handling station (Beckman-Coulter) equipped with a filtration manifold. A 652-bp segment was amplified from the 5′ region of the mitochondrial COI gene using a variety of primers (Ivanova *et al.* 2007). PCR amplifications were performed in 12.5 µl volume including 6.25 µl of 10% trehalose, 2 µl of ultra pure water, 1.25 µl of 10× PCR buffer (10mM KCl, 10mM $(NH_4)_2SO_4$, 20mM Tris-HCl (pH8.8), 2mM Mg SO_4, 0.1% Triton X-100), 0.625 µl of $MgCl_2$ (50mM), 0.125 µl of each primer (0.01mM), 0.0625 µl of each dNTP (10mM), 0.0625 µl of *Taq* DNA polymerase (New England Biolabs), and 2 µl of template DNA. The PCR conditions consisted of 94°C for 2 min, 35 cycles of 94°C for 30 s, 52°C 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The samples of larval DNA from the Galápagos Rift site were degraded and thus short-length mini-barcodes (Hajibabaei *et al.* 2006) were generated using primer set AquaF2 (unpublished) and C_FishR1t1 that spanned 183 bp of the COI barcoding sequence (Chris

Weland, pers. comm.). Since short sequences cannot be submitted to GenBank, the minibarcodes are listed here for LIDM182-07 and 183-07 respectively (both SIO 11-386):
CCCAGCCATCTCCCAATACCAGACCCCCCTCTTTGTCTGATCCGTACTAATTACAGCAGTACTACTCCT
TCTTTCCCTACCAGTTTTAGCTGCCGGAATTACAATACTTCTAACAGACCGAAATCTAAACACCACCTT
CTTCGACCCTGCCGGAGGCGGAGACCCAATCCTGTATCAGCACTTA
CCCAGCCATCTCCCAATACCAGACCCCCCTCTTTGTCTGATCCGTACTAATTACAGCAGTACTACTCCT
TCTTTCCCTACCAGTTCTGGCTGCCGGAATTACAATACTTCTAACAGACCGAAATCTAAATACCACCTT
CTTCGACCCTGCCGGAGGCGGAGACCCAATCCTGTATCAGCACTTA

Specimen information and barcode sequence data (from mtDNA COI) for this study were compiled using the Barcode of Life Data Systems (BOLD, www.barcodinglife.org; Ratnasingham & Hebert 2007). The sequence data are publicly accessible on BOLD (project LABBV) and GenBank. Sequence divergence was calculated using BOLD following the Kimura 2-parameter (K2P) model generating a mid-point rooted neighbor-joining (NJ) phenogram to provide a graphic representation of the species divergence.

The length of specimens is given as SL, measured from the median anterior end of the upper lip to the base of the caudal fin (posterior end of the hypural plate); body depth is the greatest depth from the base of the dorsal spines to ventral edge of the abdomen; body width is measured just posterior to the gill opening; head length is from the front of the upper lip to the posterior edge of the opercular flap; orbit diameter is the greatest fleshy diameter and interorbital width the least bony width; snout length is the horizontal span from the median anterior end of the upper lip to the nearest fleshy edge of the orbit; upper-jaw length is the angled distance from the median anterior end of the upper lip to the posterior end of the maxilla; caudal-peduncle depth is the least depth and caudal-peduncle length is the horizontal span between the rear base of the last anal-fin ray and the caudal-fin base; lengths of spines and rays are measured to their extreme bases; caudal-fin and pectoral-fin lengths are the length of the longest ray, pelvic-fin length is from the base of the pelvic spine to the tip of the longest soft ray. Pectoral fin-ray counts include the uppermost rudimentary ray (Randall & Allen 2010).

Sagittalarva Victor, n. gen.

Type species: *Sagittalarva inornata* (Gilbert, 1890), Baja California, Mexico; here designated.

Diagnosis. Dorsal rays IX,12; anal rays III,12; pectoral rays 13 (12 plus uppermost rudimentary ray); lateral-line continuous, inclined sharply downward below soft portion of dorsal fin, the pored scales 27, canals on scales with a single pore; head naked except for a set of small scales on each side of nape forward of the dorsal-fin origin; scales on thorax much smaller than body scales; jaws with a single pair of enlarged canine teeth at the front of the upper jaw (one tooth per side) and a single pair at the front of the lower jaw (one tooth per side) which fit between the upper pair when the mouth is closed; teeth behind enlarged canines in a regular row of caniniform to conical teeth; no posterior canine at the corner of the mouth; posterior half of upper lip with a dorsal fleshy flap, variably developed; 10+6 gill rakers, larger ones serrated and branched; snout long and pointed, snout length 3.3–3.4 in HL for fish over 70 mm SL (3.36 in holotype); body slender, depth of body 4.6–4.7 in SL for fish over 70 mm SL (4.57 in holotype, "depth 5 2/3 in length" in Gilbert (1890) must refer to TL); body very compressed, body width 9.0-10.5% SL; dorsal-fin spines pungent, first spine shortest, subsequent spines and rays progressively longer; caudal fin only slightly rounded.

Mid to late-stage larvae (7–14 mm SL) slender, flattened, and dart-shaped with marked horizontal symmetry (symmetrical above and below the lateral midline); forehead low and straight; mouth small, terminal, and at the level of the lateral midline; snout long and sharply pointed; melanophore pigment limited to the tip of the upper and lower jaws and a few small melanophores along the edge of the caudal-fin and posterior dorsal and anal-fin membranes.

Etymology. The name *Sagittalarva* derives from the combination of *sagitta*, arrow or dart in Latin, and *larva*, originally ghost or mask in Latin and first applied to immature forms by Linnaeus, referring to the unusual dart-shaped larva of the Cape Wrasse; both nouns and the combination are feminine.

Remarks. A single pair of enlarged canines at the tip of each of the upper and lower jaws with no canine at the corner of the jaw and rows of caniniform to conical teeth along the jaws (Fig. 1) separates *Sagittalarva* from all other New World julidines as well as from *Pseudojuloides*. All western Atlantic *Halichoeres* differ in having a prominent canine at the corner of the jaw and all but two have two pairs of enlarged canines in the lower jaw (*H. maculipinna* and its Brazilian sibling have only a single pair of enlarged canines at the lower jaw). Most eastern Pacific *Halichoeres* also have the canine at the corner of the jaw and the exceptions have two pairs of enlarged canines at the tip of the lower jaw (*H. notospilus* (Günther), *H. adustus* (Gilbert, 1890), and *H. insularis* Allen & Robertson, 1992) or more than a single enlarged pair at both the upper and lower jaw tips (*H. melanotis*, *H. salmofasciatus*, and *H. malpelo* Allen & Robertson, 1992; as well as *Oxyjulis californica*). Species of *Thalassoma* do not have a particularly enlarged pair of canines, while *Pseudojuloides* have flattened chisel-like incisiform teeth behind a pair of canines (Fig. 2).

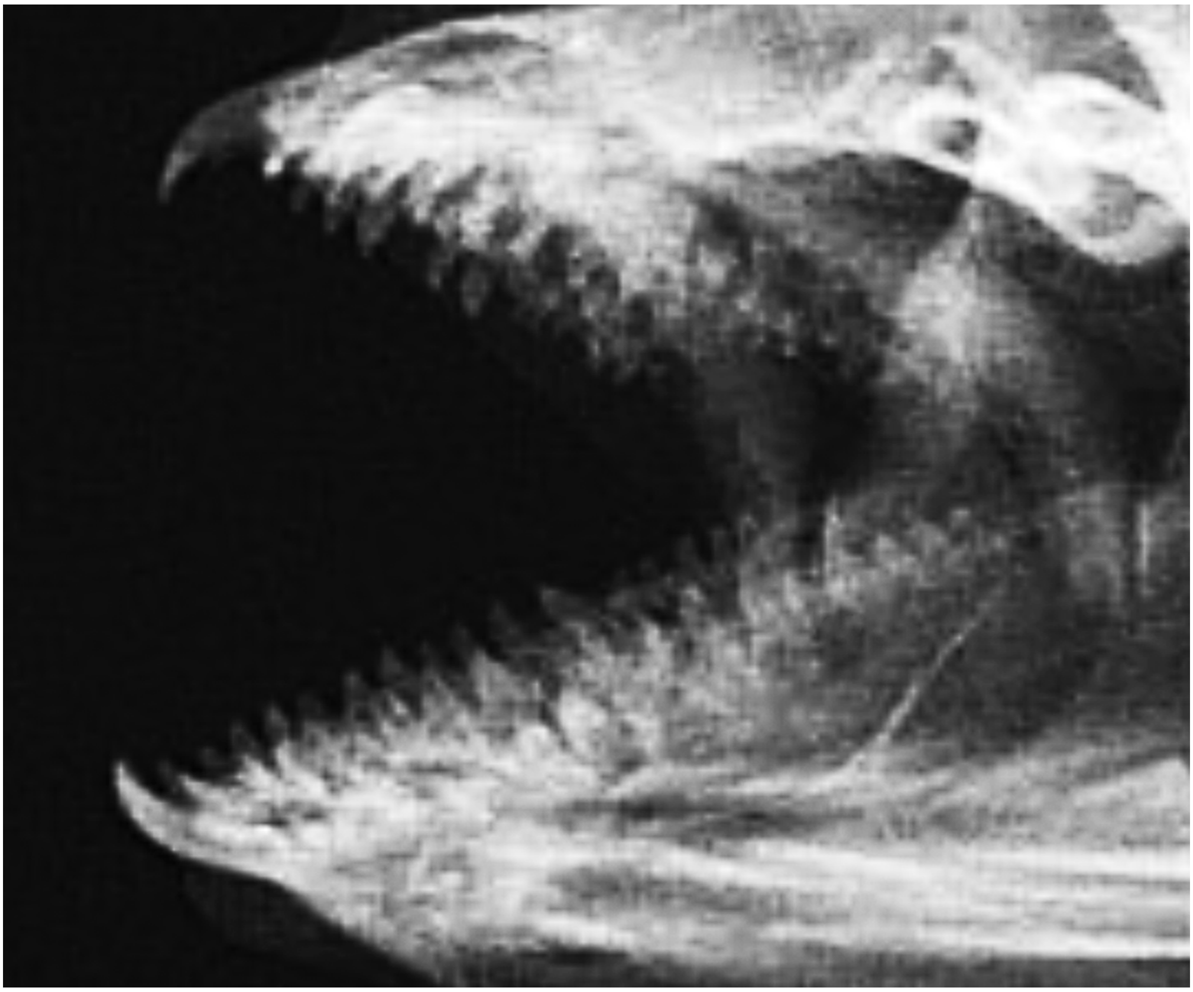

FIGURE 1. Radiograph of the head of the holotype of *Sagittalarva inornata* (USNM 44273) showing a single pair of enlarged canines at the front of the upper and lower jaws followed by rows of caniniform to coniform teeth, courtesy Sandra Raredon, USNM.

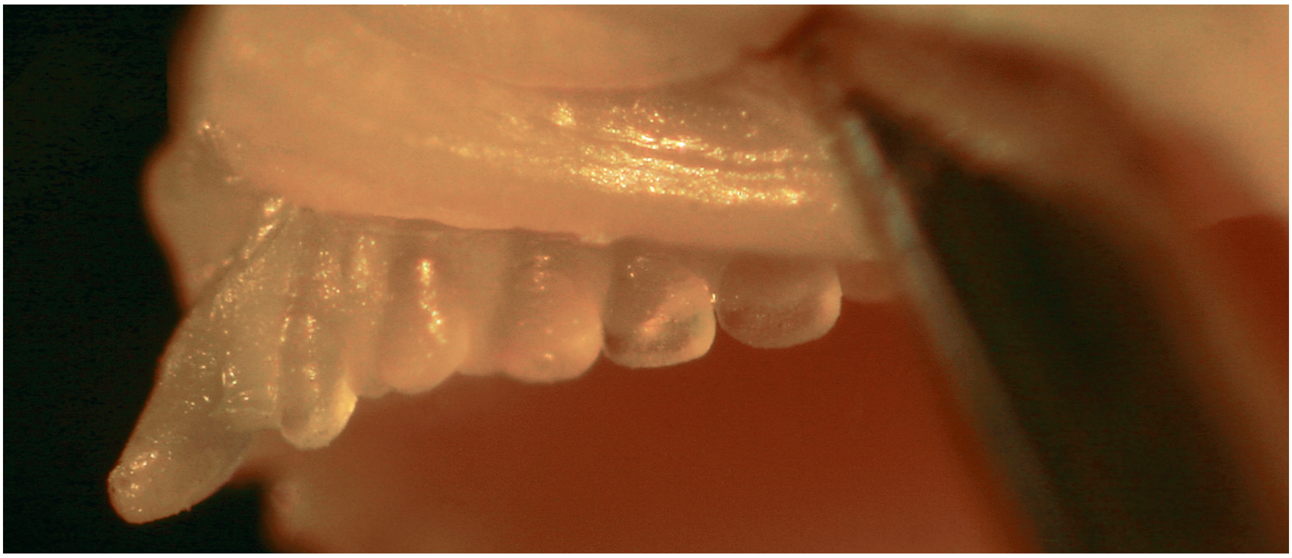

FIGURE 2. Upper jaw of *Pseudojuloides severnsi* showing spatulate incisiform teeth behind canine.

There is some ambiguity in the literature for the pectoral fin-ray counts for eastern Pacific *Halichoeres* species, with counts of 12 or 13 inconsistently cited in descriptions and guides. All of the species were examined but *H. malpelo* and they all have 13 total rays (12 plus one upper rudimentary). The species description for *H. malpelo* also reports 13 pectoral rays (Allen & Robertson 1992; 1994). In all species, the uppermost pectoral ray is a short unbranched ray, more obvious on smaller individuals and sometimes not grossly visible in adults. The next ray is typically the largest ray of the pectoral-fin series and should be counted as the second ray from the top (Randall & Allen 2010).

The larvae of New World *Halichoeres* and *Thalassoma* species differ markedly in morphology from larval *Sagittalarva inornata*. They have relatively stout bodies that are not horizontally symmetrical, relatively short snouts, and different patterns of melanophores (Watson *et al.* 1996; Beltrán-Leon & Herrera 2000; Jones *et al.* 2006; Victor 2012 and unpublished data) (Fig. 3). None have melanophores on the head and the basic complement is melanophores on the membranes between adjacent fin rays in discrete patches spaced out along the length of the dorsal and anal fins. On most larval *Halichoeres* there are three patches on the dorsal fin (front, mid, and rear) and two on the anal fin (front and rear). *Halichoeres maculipinna* larvae have only the rear patch on the dorsal and anal fins. Larval *Thalassoma* generally have very few melanophores: *T. bifasciatum* (Bloch) larvae have a patch on the membranes of the first few dorsal spines and isolated small edge melanophores along the rim of the dorsal, caudal, and anal fin membranes (these are similar to the melanophores on larval *S. inornata*). Larvae of other *Thalassoma* in the literature are typically shown with no melanophores, although the small fin-edge melanophores are present on well-preserved larvae of the eastern Pacific species and some western Pacific species as well (Victor 2012). It is likely that they occur on all *Thalassoma* larvae.

FIGURE 3. Larval labrids of the eastern Pacific Ocean: from top, *Sagittalarva inornata* 11.6 mm SL & 8.3 mm SL, *Halichoeres* 13.1 mm SL, *Thalassoma* 10.2 mm SL (identified by DNA-barcode matching).

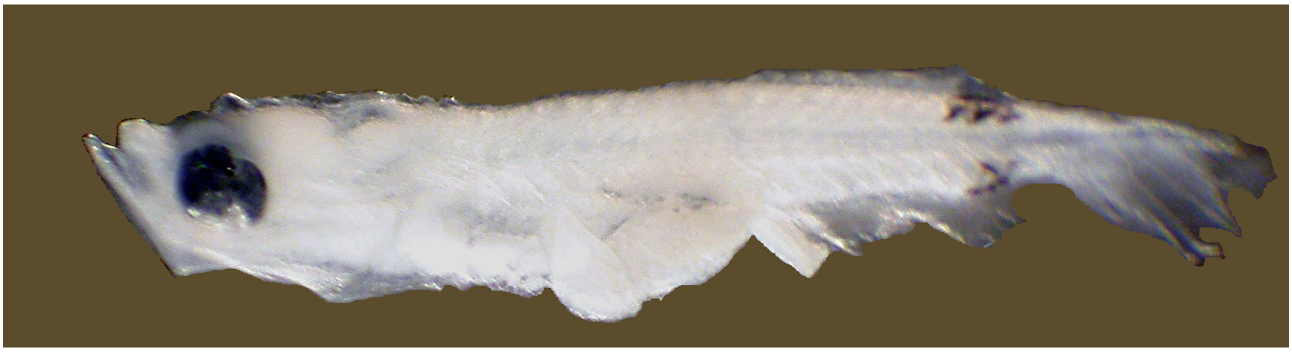

FIGURE 4. Larva of *Pseudojuloides cerasinus* from Hawaii, identified by DNA-barcode matching, courtesy David Carlon.

The larvae of *Pseudojuloides cerasinus* (Snyder) have been identified by DNA-barcode matching (collected from Hawaii by David Carlon, pers. comm. and in BOLD project FLHI) as well as by morphology (Miller *et al.* 1979, as Labrid L3, 7.2 mm SL). They differ markedly from *Sagittalarva inornata* larvae, having a short upturned snout and some very different markings from other labrid larvae, in particular distinctive internal melanophores along myomere edges within the musculature of the rear body, in addition to patches of melanophores on the membranes between the last few dorsal and anal-fin rays (Fig. 4). Bruce Mundy (pers.comm.) identified larval *P. cerasinus* in his Hawaiian collections and found both the internal and fin-membrane melanophores as well as additional melanophores over the cranium and a complex pattern of erythrophores (usually lost in preservation) on fresh late-stage *P. cerasinus* larvae.

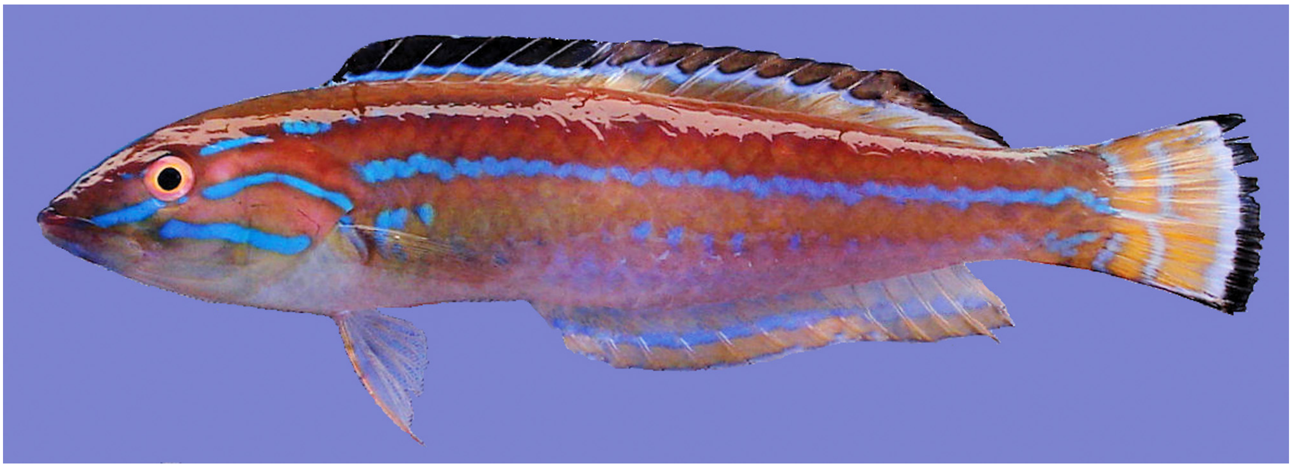

FIGURE 5. *Sagittalarva inornata*, male, 136 mm SL, SIO 07-94, photograph by John Snow.

Sagittalarva inornata (Gilbert, 1890), n. gen., n. comb.

(Figs. 5–9)

Pseudojulis inornatus Gilbert, 1890: 67 (type locality, 22.87°N, 109.92°W; Cabo San Lucas, Baja California, Mexico).
Pseudojulis sp. Victor, 1987: 146, Fig. 1 (0.81°N, 86.23°W; 400 km E. of Galápagos).
Halichoeres cf. *malpelo* Beltrán-Leon & Herrera, 2000: 524, Fig. 181 (continental coastline of Colombia).
Halichoeres raisneri Baldwin & McCosker, 2001: 89, figs. 4–5 (type locality, 1.39°N, 91.81°W; Wolf Island, Galápagos, Ecuador).
Halichoeres cf. *raisneri* Bussing & Lopez, 2004: 120 (5.57°N, 87.03°W; Cocos Island, Costa Rica).
Halichoeres sp. Bussing & Lopez, 2004: 121, figure (5.57°N, 87.03°W; Cocos Island, Costa Rica).

Material examined. USNM 44273, 73 mm SL, Cabo San Lucas, Baja California, Mexico, 22.87°N, 109.92°W, *Albatross*, dredge, 31 fathoms, 1 May 1888 (holotype of *Pseudojulis inornatus*); USNM 357795, 90 mm SL, SE Wolf Island, Galápagos Islands, 1.39°N, 91.81°W, submersible JSL-II-sta. 3087, 115m, C. Baldwin & J.

Gomezjurado, 22 June 1998 (holotype of *Halichoeres raisneri*); CAS 209810, 2: 39.8–78.8 mm SL, 1.39°N, 91.81°W, submersible JSL-II-sta. 3086, 114–125m, C. Baldwin & J. McCosker, 22 June 1998 (paratypes of *Halichoeres raisneri*); SIO 65-250, 106 mm SL, Isla Cerralvo Channel, Baja California, Mexico, 24.19°N, 109.99°W, *Horizon*, trawl, up to 46 fathoms, 2 July 1965; SIO 07–34, 162 mm SL, Gordo Bank, San Jose del Cabo, Baja California, Mexico, 23.02°N, 109.50°W, hook and line, 50–100m, John Snow, 15 February 2007; SIO 07-94, 136 mm SL, San Luis Canyon, San Jose del Cabo, Baja California, Mexico 23.05°N, 109.40°W, hook and line, 50–100m, John Snow, 21 July 2007; SIO 11-386, 31: 7.0–14.0 mm SL, open ocean 400 km E. of Galápagos Islands, 0.81°N, 86.23°W, plankton net, 0–100m, B. Victor, 10 March 1985.

Diagnosis. Dorsal rays IX,12; anal rays III,12; pectoral rays 13 (12 plus uppermost rudimentary ray); lateral-line continuous, inclined sharply downward below soft portion of dorsal fin, the pored scales 27, canals on scales with a single pore; head naked except for a set of small scales on each side of nape forward of the dorsal-fin origin; scales on thorax much smaller than body scales; jaws with a single pair of enlarged canine teeth at the front of the upper jaw (one tooth per side) and a single pair at the front of the lower jaw (one tooth per side) which fit between the upper pair when the mouth is closed; teeth behind enlarged canines in a regular row of caniniform to conical teeth; no posterior canine at the corner of the mouth; posterior half of upper lip with a dorsal fleshy flap, variably developed; 10+6 gill rakers, larger ones serrated and branched; snout long and pointed, snout length 3.3–3.4 in HL for fish over 70 mm SL (3.36 in holotype); body slender, depth of body 4.6–4.7 in SL for fish over 70 mm SL (4.57 in holotype, "depth 5 2/3 in length" in Gilbert (1890) must refer to TL); body very compressed, body width 9.0-10.5% SL; dorsal-fin spines pungent, first spine shortest, subsequent spines and rays progressively longer; caudal fin only slightly rounded.

Mid to late-stage larvae (7–14 mm SL) slender, flattened, and dart-shaped with marked horizontal symmetry (symmetrical above and below the lateral midline); forehead low and straight; mouth small, terminal, and at the level of the lateral midline; snout long and sharply pointed; melanophore pigment limited to the tip of the upper and lower jaws and a few small melanophores along the edge of the caudal-fin and posterior dorsal and anal-fin membranes (Fig. 3).

Description. Dorsal rays IX,12; anal rays III,12; all dorsal and anal rays branched, the last to base; pectoral rays 13 (12 plus uppermost rudimentary ray); pelvic rays I,5; principal caudal rays 14, median 12 branched, upper procurrent rays 6, lower procurrent rays 5, last procurrent ray segmented; lateral-line continuous, inclined sharply downward below soft portion of dorsal fin, the pored scales 26 plus one pored scale on caudal fin base; canals on scales with a single pore; gill rakers 10 on lower limb including angle, 6 on upper limb, larger gill rakers serrated and branched, some antler-like; branchiostegal rays 5; vertebrae 25.

Body proportions as % of SL for 162 mm male (136 mm SL male in parentheses). Body long and slim, maximum body depth 21.6 (21.3)% or 4.6 in SL (4.7); body very compressed, width 10.5 (9.0)% or 2.1 in depth (2.4); head length 31.5 (31.1)% or 3.2 in SL (3.2); snout long, length 9.6 (9.0)% or 3.3 in HL (3.4); orbit diameter 5.6 (5.1)% or 5.7 in HL (6.0); interorbital width 5.6 (4.7)% or 5.7 in HL (6.6); caudal peduncle narrow, depth 10.5 (11.8)% or 3.0 in HL (2.6); caudal-peduncle length 11.7 (11.8)% or 2.7 in HL (2.6).

Mouth small, terminal, oblique at about 45 degrees, upper-jaw length 8.0 (7.9)% or 3.9 in HL (3.9); jaws with a single pair of enlarged canine teeth at the front of the upper jaw (one tooth per side) and a single pair at the front of the lower jaw (one tooth per side) which fit between the upper pair when the mouth is closed; teeth behind enlarged canines in a regular row of 10-20 caniniform to conical teeth in each quadrant; no posterior canine at the corner of the mouth; pharyngeal dentition described in Baldwin and McCosker (2001); posterior half of upper lip with a dorsal fleshy flap (Fig. 6), variably developed on smaller males and females, lower lip with a curved labial flap; preopercular margin free and smooth on the vertical portion from the lowest blue cheek stripe down and on the horizontal portion forward to a level in front of the eye; a single line of suborbital pores, 7 in semicircle from below posterior nostril to behind upper-pupil level. Scales cycloid and large, head naked but smaller scales extending forward of the dorsal fin origin and thorax with progressively smaller scales.

Origin of dorsal fin above first lateral-line scale, predorsal length 26.5 (23.5)% or 3.8 in SL (4.3); dorsal-fin spines slender but pungent, progressively longer, first spine 6.4%, second 7.0%, third 7.3%, last 7.9%; longest dorsal-fin ray 10.5%; dorsal-fin base length 59.9 (66.2)%; preanal length 54.3 (52.9)% or 1.8 in SL (1.9); first anal-fin spine slender and short 3.7%, second 6.2%, third 7.6%; longest anal-fin ray 11.0%; anal-fin base length 33.9 (34.6)%; caudal fin very slightly rounded, length 16.0 (16.9)% or 2.0 in HL (1.8); third pectoral ray longest 16.7 (18.4)% or 1.9 in HL (1.7); pelvic fins long 19.1 (17.6)% or 1.7 in HL (1.8); prepelvic length 27.8 (28.7)% or 3.6 in SL (3.5).

FIGURE 6. Upper lip flap on male *Sagittalarva inornata*, 136 mm SL, SIO 07-94.

Color in life of male primarily salmon pink to orange with bright blue stripes (Fig. 5). The stripes comprise a mid-lateral blue line along the body, starting behind the head, curving up along the pectoral-fin base then running straight to the caudal-fin base; a broken or partial blue stripe along the lower lateral body; a blue stripe down the sagittal midline of the head; and three blue stripes across each side of the head: one behind the upper eye running back to below the anterior spinous dorsal fin, a second rearward from the upper jaw passing interrupted at the lower rim of the orbit and curving down across the operculum to meet the upper edge of the pectoral fin base, and the lowest from just behind the corner of the jaw across the lower operculum. Similar blue stripes run along the dorsal and anal fins about mid-way out on the membranes and, to a lesser degree, along the edge of the membranes. There are lighter contrasting stripes of light pinkish yellow running above the blue stripe on the snout and operculum and continuing above and below the mid-lateral blue line (more obvious in females, especially underwater photos).

There is a prominent contrasting black-to-dark-brown ribbon along the outer part of the dorsal fin as well as a sharp black edge to the caudal fin, that notably extends forward along the edges of the upper and lower tips of the tail. The caudal fin is bright orange with three thin blue-white bars, the last highlighting the black fin edge. There is a small dark spot on the dorsal midline of the body just after the base of the last dorsal fin ray.

Based on the color photograph in Baldwin and McCosker (2001), females have the same basic marking pattern, but without the contrasting black markings. The blue stripes are less intense, and appear pinkish blue and are adjacent to bright orange-yellow background stripes that are more distinct than on the males. In underwater photographs, the blue or pink stripes are mostly light streaks and the yellow stripes are intense (Fig. 7). The stripes are broken by five pale indistinct bars producing a pattern of stripes and bars on living fish (Fig. 7 and 8), but not obvious on dead or preserved specimens.

Juveniles in life have the same basic stripe pattern on a light background but with the upper-body dark stripe more contrasting. There is a prominent black spot at the base of the mid-portion of the soft dorsal fin that stands out in underwater videos (Fig. 8). The juveniles also show the five light bars and the upper-body dark stripes from each side meet at the end of the dorsal fin followed by the last light bar, highlighting the junction as a dark blotch.

FIGURE 7. Underwater photograph of female *S. inornata* in Baja California from videos by Octavio Aburto-Oropeza and Brad Erisman.

FIGURE 8. Underwater photograph of juvenile *S. inornata* in Baja California from videos by Octavio Aburto-Oropeza and Brad Erisman.

Color of male in alcohol pale yellow-brown with the blue lines mostly grey and outlined in dark and the black markings remaining prominent. Notably, there is a small but distinct dark spot on the body just behind the insertion of the last dorsal-fin ray. Females have no distinctive markings in preservative, but smaller individuals show the dark spot on the base of the fifth dorsal-fin soft ray (Baldwin & McCosker 2001).

Mid to late-stage larvae (7–14 mm SL) slender, flattened, and dart-shaped with marked horizontal symmetry (symmetrical above and below the lateral midline); forehead low and straight; mouth small, terminal, and at the level of the lateral midline; snout long and sharply pointed; melanophore pigment limited to the tip of the upper and lower jaws and a few small melanophores along the edge of the caudal-fin and posterior dorsal and anal-fin membranes (Fig. 3).

Discussion

The taxonomic history of *Sagittalarva inornata* has been complicated. Gilbert (1890) described *Pseudojulis inornatus* from a single apparently immature 72 mm fish (USNM 44273, Fig. 9) in a short half-page of his report on the Pacific Coast collections from the vessel *Albatross*. The specimen was dredged from 31 fathoms (about 60m) off Cabo San Lucas in Baja California (*Albatross* station 2829; 22.87°N, 109.92°W). His brief description noted a “very slender” body, a single pair of enlarged canines at the tip of the each jaw (upper and lower), and no posterior canines at the corner of the jaw. His color description was limited and bland, leading to the now obviously inappropriate Latin name of *inornatus* (meaning unadorned). He described dark stripes near the base of the dorsal fin with a black spot at the base of the fifth dorsal-fin ray, presumably the fifth segmented ray (which can be seen on the holotype only as a dark streak along the shaft of the ray) and another black spot at the base of the last dorsal-fin ray. Unfortunately, his dorsal fin-ray count of IX,11 is an error and the x-ray of the holotype shows IX,12 (Fig. 9).

Subsequently, a second deepwater julidine wrasse was described in 2001 from the eastern Pacific as *Halichoeres raisneri*, based on three juvenile and female specimens (39.8 to 90 mm SL) captured on deep slopes off the Galápagos Islands by Baldwin and McCosker (2001). The species was noted to be a slender wrasse with a long pointed snout, IX,12 dorsal-fin rays, a dark spot at the base of the fifth soft dorsal-fin ray, a single pair of enlarged canines at the tip of each jaw (upper and lower) and no posterior canines. Despite the similarity, and probably due to the distance from Baja California, *H. raisneri* was not compared with *Pseudojuloides inornatus*, as it was known at the time. In the description of *H. raisneri* it was noted, however, to not share the incisiform teeth of *Pseudojuloides cerasinus* from the Indo-Pacific.

FIGURE 9. Photograph and radiograph of the holotype of *Sagittalarva inornata*, 73 mm SL, USNM 44273, courtesy Sandra Raredon, USNM.

Just before the final publication of *Halichoeres raisneri*, a 143 mm SL male wrasse was discovered from Cocos Island that shared the slender body, long snout, and IX,12 dorsal fin-ray count, as well as the fleshy flap on the upper lip characteristic of *H. raisneri* (drawing in Bussing and Lopez (2005)). This adult male was preserved and lost color before examination but is illustrated with the same head stripes and black tail margin as the male specimens from Baja California described here. Baldwin and McCosker (2001) noted that the Cocos male shared most of the diagnostic features of *H. raisneri*, but did not designate the specimen as type material since it was captured at a distant location and was a male while the types were females. In the most recent review of Galápagos fishes by McCosker and Rosenblatt (2010), the range of *H. raisneri* has been extended to Cocos and Malpelo Island based on the male of Bussing and Lopez (2005), as well as underwater video showing wrasses with the characteristic markings of *H. raisneri* occurring in deep water off all three islands.

Deepwater videos taken by Octavio Aburto-Oropeza and Brad Erisman on a series of dives in the submersible DeepSee in 2008 off the tip of Baja California show wrasses with the same appearance (Fig. 7) as the underwater photograph of *H. raisneri* from Galápagos shown in Fig. 4 of Baldwin and McCosker (2001). On close examination of the videos, smaller individuals can be seen to have the dark stripes near the base of the dorsal fin and the prominent dark spot at the base of the mid soft-dorsal fin described by Gilbert (1890) for *Pseudojulis inornatus* (Fig. 8). The dark stripes meet in a dark patch at the end of the dorsal fin in the area described as a dark spot in the original description. The wrasses are reported to be common off the tip of Baja California at the 80–150 m depth surveyed (Brad Erisman, pers. comm.).

On subsequent review of the collections at SIO, a small (106 mm SL), presumably male, specimen of *S. inornata* was found collected in 1965 from near the tip of Baja California with a deep trawl (up to 46 fathoms) and labeled as *Pseudojulis* sp. (SIO 65-250). The specimen has few markings remaining, but does share the diagnostic markings of a dark edge to the caudal fin and a dark spot on the dorsal midline near the base of the last dorsal-fin ray, as well as a relatively small (and inconspicuous) upper jaw flap.

Before the discovery of *H. raisneri*, one of us (BV) had described a series of 31 unusual labrid larvae from the open ocean over the Galápagos Rift hydrothermal vents, midway between the offshore islands: about 400 km east of the Galápagos Islands, 500 km south of Cocos Island, and about 600 km west of Malpelo Island and the continent (Victor 1987). They were described as particularly slender labrid larvae with a long pointed snout and having a IX,12 dorsal fin-ray count and were labeled as *Pseudojulis* sp., with the proviso that their otolith morphology did not match that of known *Pseudojulis* species (Victor 1987). It is notable that the larvae were found to be up to 83 days old, a long pelagic larval phase for most reef fishes and a period certainly capable of transporting larvae between regions in the eastern Pacific, if not across the entire Indo-Pacific Ocean (Robertson *et al.* 2004).

Beltrán-Leon and Herrera (2000) surveyed the larval fishes of the Colombian Pacific coast and reported seven of the same larvae and illustrated one (15.6 mm SL) showing the characteristic melanophores at the tip of the jaws and along the edges of the membranes of the rear dorsal, caudal, anal, and pelvic fins. Since labrid larvae cannot be identified to species by meristics, they provisionally assigned the larvae to *Halichoeres* cf. *malpelo*, the endemic representative of the *H. melanotis* complex on Malpelo Island (Allen & Robertson 1992). Although there are no DNA sequences available for *H. malpelo*, populations of *H. melanotis* from the continental coast and *H. salmofasciatus* from Cocos Island share a COI mtDNA clade far distant from that of *Sagittalarva inornata* (Fig. 10).

The final step in the chain of deduction was to link the deepwater labrids found in Baja California with those collected in the southern offshore islands and the adults with the unusual larval form. The barcode DNA sequence of the male specimen from Baja was a 98.9% and 98.3% match to minibarcode sequences for two of of the series of labrid larvae from over the Galápagos Rift hydrothermal vents (GenBank accession number for SIO 07-94 is JQ952620; minibarcodes in Methods section (and not GenBank eligible)). The degree of match is within the range of intraspecific variation for the barcode sequence for the labrids and the match is likely closer for the full barcode sequence since artifactual variation can be introduced by sequencing “minibarcodes” on partially degraded DNA.

In summary, after the true fin-ray count and dentition of the holotype named *Pseudojulis inornatus* are established, it becomes evident that 1) female *Halichoeres raisneri* from Galápagos have the same markings, fin-ray counts, dentition, body shape, size, and deep-slope habitat of the holotype named *Pseudojulis inornatus*; 2) the male specimens from Baja California have the same upper-lip flap, markings, morphology, and meristics as the male *H. raisneri* from Cocos Island, in addition to the dark spot behind the last dorsal-fin ray described for *Pseudojulis inornatus*; 3) underwater images of the Baja wrasses show the markings described for both *Pseudojulis inornatus* and *H. raisneri*; 4) the larvae from over the Galápagos Rift hydrothermal vents match the DNA sequence of the Baja males; and 5) both the Baja specimens and underwater videos are from the same location and depth in Baja as the holotype of *Pseudojulis inornatus*. On the basis of these findings it can be concluded that these collections are all various life stages of the same species.

An almost exactly analogous case of a recent new species discovery in the Panamic region of the eastern Pacific proving to be a synonym of a century-old species description from the tip of Baja California (and also a fish rarely collected and at the time never photographed) is the Cape Razorfish *Xyrichtys mundiceps* Gill. The species *Xyrichtys perlas* Wellington, Allen & Robertson, 1994 was described from the Perlas Islands off Panama, also from a few juveniles and females. However, when *X. mundiceps* were finally captured and photographed in Baja California, there proved to be no difference between the description of *X. perlas* and the females of *X. mundiceps* (Victor *et al.* 2001). In a further remarkable extension of the congruence, larval razorfish from the collection over the Galápagos Rift hydrothermal vents proved to be a 99.85% match to adult *X. mundiceps* collected at the tip of Baja California, extending the range of the species over 3,500 km to the south of Baja (GenBank numbers JQ839662 (larval) and JQ839664 (adult)). Razorfishes of *Xyrichtys* are also known to have very long larval lives of two months or more (Victor 1986, Victor & Wellington 2000) and, in the case of the pelagic Galápagos Rift larvae, reaching up to 130 days (Victor 1987).

There are old reports of sightings of *Pseudojuloides cerasinus* in the eastern Pacific (Allen & Robertson 1994), however no specimens have been collected and those are likely mistaken Cape Wrasses and the listing has not been continued in more recent guides (Robertson & Allen 2008).

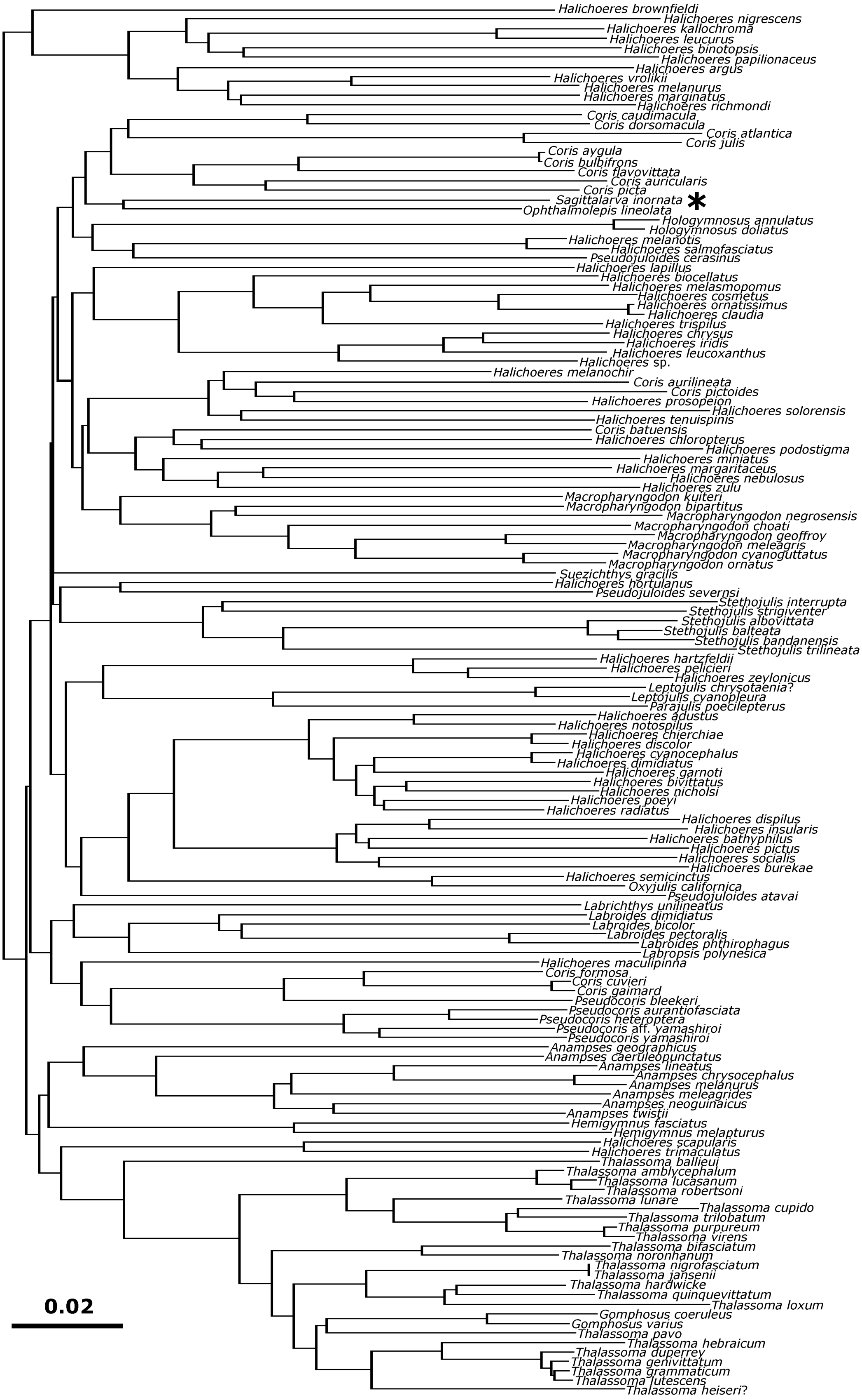

FIGURE 10. A phenetic neighbor-joining tree based on the COI mtDNA sequences of 146 julidine wrasses, including *Sagittalarva inornata* (with asterisk), following the Kimura two-parameter model (K2P) generated by BOLD. The GenBank accession numbers corresponding to the sequences in the tree are listed in Appendix 1. The scale bar at lower left represents a 2% sequence difference.

Phylogenetics. The phenetic neighbor-joining tree based on the mtDNA COI segment includes a large number and wide variety of julidines that are presently in the BOLD barcode database (Fig. 10). The database now includes about 60 of the 80 known species of *Halichoeres* (Parenti & Randall 2011) and about 150 of the approximately 200 julidines. The definition of the julidine tribe has been in flux over the years and we include the labrichthyines which nest within other julidines and exclude the pseudolabrines which are a monophyletic group sister to all other julidines (Westneat & Alfaro 2005).

The phenetic tree is primarily used to compare an unknown sequence to all known sequences for identification of a nearest neighbor, i.e. the closest sequence in a clustering model (Ratnasingham & Hebert 2007). This does not always yield the same result as the closest sequence in a simple similarity measure (as in the BLAST similarity model used by GenBank) or a probability-based model (such as the Hidden Markov model used in BOLD in the ID engine for fishes). In other words, with a large number of comparisons, two sequences can have the same percent similarity in the sequence to a test sequence but may or may not cluster on the same branch of the tree assembled with a neighbor-joining model.

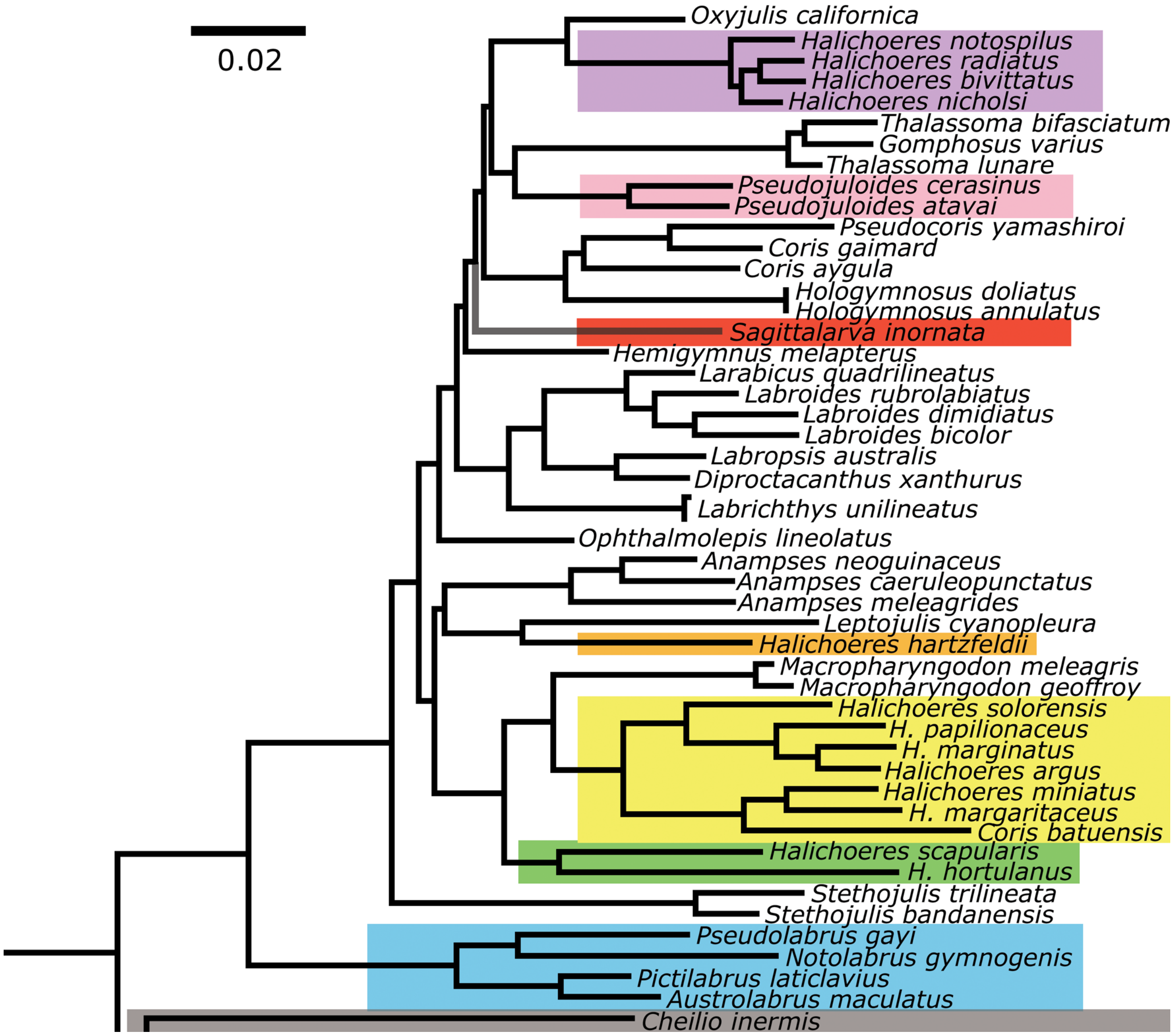

FIGURE 11. Julidine portion of the phylogenetic tree from Westneat and Alfaro (2005), with the newly-available sequences for *Sagittalarva inornata* added, based on a maximum-likelihood model utilizing nuclear genes Rag2 and Tmo4c4 and mitochondrial genes 12s and 16s (log likelihood = -47392.14). *S. inornata* is highlighted in red, the New World *Halichoeres* clade in purple, a large Indo-Pacific *Halichoeres* clade in yellow, smaller Indo-Pacific clades of *Halichoeres* in orange and green, *Pseudojuloides* in pink, the "julidinoid" Pseudolabrines in blue and *Cheilio* and other non-julidines below. The scale bar at upper left represents 0.02 substitutions per site. The sequences for Rag2, Tmo4c4, 12s, and 16s for *S. inornata* correspond to GenBank accessions JX684103–6; the accession numbers for the remaining sequences are listed in Table 1 of Westneat and Alfaro (2005).

The barcode sequence of *Sagittalarva inornata* is not close to any of the other julidines sequenced, with the most similar sequence more than 12% different (in the BOLD ID engine) and the genus of the nearest neighbor (in a neighbor-joining tree) not consistent, varying depending on the selection or number of comparators one chooses. The pattern is characteristic of neighbor-joining trees containing deeply divergent lineages where the deep branching points are not robust and typically sensitive to changes in the number and suite of comparators. In fact, 10 species in 5 julidine genera are about the same distance from *S. inornata*, i.e. between 86% and 87.3% "similarity percentage" in the BOLD ID engine. When compared with the full BOLD database of all fishes (by the BOLD ID engine in July 2012, against more than 70,000 sequences), the "most similar" sequence is 87.3%, to *Halichoeres tenuispinis* (Günther) from Korea. However, in the neighbor-joining tree of 146 julidines in Fig. 10, the nearest-neighbor lineage is *Ophthalmolepis lineolata* (Valenciennes), the Maori Wrasse of temperate South Australia (86.9% in BOLD ID engine similarity percentage; but it is important again to note that the highest "similarity percentage" is not automatically the "nearest neighbor" in a clustering model).

In contrast to the one-gene phenetic tree derived from neighbor-joining, a phylogenetic tree using multiple nuclear and mitochondrial genes provides a more powerful assessment of phylogenetic relationships. Indeed, Westneat and Alfaro (2005) used two nuclear (Rag2 and Tmo4c4) and two mitochondrial (12s and 16s) genes for a maximum-likelihood analysis of labrid relationships. Their study included fewer julidine species but has more phylogenetic resolution than a tree based on a single gene. When the newly available sequences for *Sagittalarva inornata* are added into their analysis (new log likelihood = -47392.14; Fig. 11), *Sagittalarva inornata* falls well within the julidine labrids, but not in or close to other clades of julidines and is not associated with either *Pseudojuloides* or the New World *Halichoeres*.

There has been much discussion in the literature of the difficulties in resolving evolutionary relationships among the julidine labrids (Parenti & Randall 2000, Rocha 2004, Barber & Bellwood 2005, Westneat & Alfaro 2005, Kuiter 2010). The present arrangement of genera does not always reflect the genetic relatedness within the julidine labrids and no consensus exists on the best way to resolve the issue . Genetic lineages may be somewhat consistent in consolidating groups of species (Kuiter (2010) provides a proposed reshuffling), but finding confirmatory synapomorphies or diagnostic morphologic or meristic correlates for many groupings remains elusive.

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