



Eusociality unveiled: discovery and documentation of two new eusocial shrimp species (Caridea: Alpheidae) from the Western Indian Ocean

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Abstract

The alpheid snapping shrimp genus *Synalpheus* Spence Bate, 1888, is a prominent component of arthropod diversity found in coral reefs. Notably, *Synalpheus* is the only genus of marine organisms known to exhibit eusocial behavior. Although eusociality has evolved at least four times independently in *Synalpheus*, all described eusocial species are from the West Atlantic, with only a single study documenting possibly eusocial species from Indonesia. In 2008, during an expedition to Madagascar organized by the Florida Museum of Natural History (FLMNH), a diverse array of sponge-dwelling species was collected, including two species of *Synalpheus* exhibiting colonial behavior. Through detailed examination of these specimens, we have confirmed that these two species are eusocial and represent new eusocial species of *Synalpheus* outside of the West Atlantic. Consequently, we provide the first official documentation of eusocial species from the Western Indian Ocean and present their taxonomic descriptions and their phylogenetic relationships with other species of the genus in this study.

Keywords

Synalpheus, phylogeny, sponge-dwelling, Madagascar, new species, *S. gustavi* sp. nov., *S. sponjy* sp. nov.

1. Introduction

The snapping shrimp in the genus *Synalpheus* Spence Bate, 1888, comprise a major component of the crustacean diversity within coral reefs (Coutière 1909; Banner and Banner 1975, Chace 1988, Anker et al. 2012; Anker and De Grave 2016). Remarkably, it stands as the sole genus among all marine animals to exhibit eusocial living systems (Duffy 1996, 2007). Eusociality was first discovered by Duffy in 1996, and since then, multiple

occurrences of eusociality have been documented for several species in the Western Atlantic (Ríos and Duffy 2007; Hultgren et al. 2011; Hultgren and Brandt 2015; Ashrafi and Hultgren 2022). Duffy (2007) identified several key traits that define eusocial *Synalpheus* shrimps. These include the presence of colonies consisting of multiple generations with pronounced reproductive skew and division of labor, such as cooperative defence and

cooperative care of offspring. All eusocial species (in which larvae have been characterized) also have exclusively direct development. That is, eggs hatch directly into crawling juveniles who tend to stay in the sponge, instead of planktonic larvae, allowing for the establishment of multi-generational colonies within the same host sponge. Eusocial species typically have a reduced number of eggs produced by the queen and larger egg size (Duffy 1996, 2007; Hultgren et al. 2021), relative to species with planktonic larvae who tend to have smaller and more numerous eggs.

Extensive research has delved into various aspects of this unique lifestyle, encompassing colony composition (Tóth and Bauer 2007; Duffy and Macdonald 2009; Chak et al. 2015), interactions between shrimps and sponges (Duffy et al. 2002; Tóth and Duffy 2008; Hultgren and Duffy 2010), phylogenetic relationships (Hultgren and Duffy 2011, for instance), and documentation of larger genomes in eusocial species (Jeffery et al. 2016; Chak et al. 2021; Hultgren et al. 2021). However, eusociality has been primarily studied in West-Atlantic species of *Synalpheus*, while little attention has been given to eusociality in other regions, such as the Indian Ocean and Pacific Ocean, with the exception of a single study conducted by Didderen et al. in 2006. This lack of exploration is primarily attributed to the fact that taxonomic studies on Indo-Pacific *Synalpheus* species were conducted before Duffy's groundbreaking work in 1996 (instances of important taxonomic work in the Indo-West Pacific: Coutière 1905, 1909, 1921; De Man 1910, 1911; AH Banner 1959; Banner and Banner 1975, 1981, 1983). Nonetheless, traces of eusociality can be found in earlier studies, where skewed sex ratios were noted (Banner and Banner 1975; Banner and Banner 1981, 1983) and species were even named based on this distinct feature (e.g. *S. paradoxus* Banner and Banner, 1981).

Didderen et al. (2006) documented extremely large colonies (40–372 individuals) of males (non-ovigerous) individuals, with often only 1 or 0 obviously ovigerous females, in *Synalpheus neptunus neptunus* (Dana, 1852), living in sponges in Indonesia. They also documented large colonies of *S. fossor* (Paulson, 1875), *S. hastilicrassus* Coutière, 1905, and *S. aff. neomeris* (De Man, 1897), living in sponges from Indonesia. However, these species are also often collected as heterosexual pairs from other localities (i.e., Moorea, Guam, Papua New Guinea, New Caledonia, Australia, Madagascar, and Red Sea), and genetic data suggests many of these species represent cryptic species complexes (Hultgren et al. 2014; Hultgren, Ashrafi, unpublished data). Interestingly, Banner and Banner (1975) reported a colony of *S. neptunus* (Dana, 1852) consisting of a colony of 44 individuals with no females, which they designated as the subspecies *S. neptunus germanus* Banner and Banner, 1975. Genetic evidence suggests *S. neptunus* is a cryptic species complex with several different related species (Hultgren et al. 2014), and several specimens of *S. neptunus* are found alone or in heterosexual pairs, suggesting at least some of the cryptic species may exhibit a pair-living lifestyle. A thorough taxonomic revision of these cryptic species

complexes—*S. hastilicrassus*, *S. fossor*, *S. aff. neomeris*, and *S. neptunus*—is needed to properly document the extent of eusociality in these Indo-West Pacific species.

In 2008, a comprehensive expedition to Madagascar was organized by the Florida Museum of Natural History (FLMNH: Gainesville, Florida, USA). The primary objective of this expedition was to study the biodiversity of the region. During this expedition, a substantial number of *Synalpheus* specimens were collected. In 2022, HA visited the collection and carefully studied the specimens belonging to the genus *Synalpheus*.

Notably, the expedition to Madagascar yielded a significant number of cryptic sponges, which were frequently sampled. This fortuitous circumstance provided an ample opportunity to study the *Synalpheus* species associated with these sponges. Among the inhabitants of the sponges, two distinct species of *Synalpheus* stood out, with each species represented by a substantial number of specimens collected from a single sponge. Interestingly, within each sponge, only a single ovigerous female was found, if any.

By conducting a meticulous examination of both species, it became evident that they had developed a eusocial living system, marking a remarkable discovery previously undocumented in the Western Indian Ocean. These two previously unknown species are now being formally described and have been incorporated into a multi-gene phylogenetic tree. This inclusion allows for the reconstruction of how many times eusociality has independently evolved outside of the Western Atlantic *Synalpheus* species.

2. Material and Methods

2.1. Phylogenetic Tree and taxon sampling

The materials for the present study were collected during several expeditions conducted by the Florida Museum of Natural History (FLMNH), Gainesville, Florida. These expeditions include Madagascar 2008, French Polynesia/Moorea 2008, Western Australia 2009, Guam 2010, and Saudi Arabia 2013.

The most recent comprehensive worldwide tree of *Synalpheus* (Hultgren et al. 2014) featured *S. gustavi* **sp. nov.** as an undescribed species (referred to as *S. aff. brevifrons*). However, *S. sponjy* **sp. nov.** was not included. To augment the existing tree, we sequenced multiple representatives of these species, and used existing data, from four genes: mitochondrial COI DNA (cytochrome oxidase I), mitochondrial 16S rRNA, 18S rRNA, and the nuclear PEPCK gene (phosphoenolpyruvate carboxylase). Additionally, we extracted and sequenced DNA from museum specimens of several *Synalpheus* species (e.g. *S. theano*, *S. sladeni*, and *S. anceps*) that exhibit several morphological similarities to the new species, but were not included in the Hultgren et al. (2014) tree.

Table 1. All species sequenced for phylogenetic analyses used in this study, including the genetics # (referenced in phylogenetic trees), locality, collection number, and GenBank accession numbers. For the complete list of the abbreviations used in the voucher numbers, see Supplementary data 1 in Hultgren et al. (2014). GenBank numbers in bold indicate sequences used in consensus tree.

Species	Genetics#	Locality	Collection#	COI	PEPCK	16S	18S
<i>Synalpheus africanus</i>	592	São Tomé	AA--06-215	KJ595031	KJ625140	KJ625020	
<i>Synalpheus</i> cf. <i>africanus</i> B	595	Sao Tome	OUMNH.ZC.2012-07-130	KJ595053	KJ625142	KJ595185	KJ595255
<i>Synalpheus agelas</i>	459	Belize	CBC09-1508	KJ595032		HQ435419	KJ595245
<i>Synalpheus anceps</i>	2182	Guam	FLMNH-UF 37335	OR482981		OR483005	OR483030
<i>Synalpheus androsi</i>	467	Belize	CBC09-6502	KJ625033		HQ435421	KJ595246
<i>Synalpheus ankeri</i>	477	Panama	P09-6903	KJ625041	KJ625069	HQ435473	KJ595242
<i>Synalpheus apioceros</i>	593	Panama	RMNH.CRUST.D.54889	KJ595035	KJ625141	KJ595177	KJ595247
<i>Synalpheus bannerorum</i>	563, 634	Panama	OUMNH.ZC.2014-04-027	KJ595036		KJ595178	
<i>Synalpheus belizensis</i>	250	Barbados	BR08-6901	KJ595038			KU682627
<i>Synalpheus bituberculatus</i>	1290	Singapore	OUMNH.ZC.2014-11-190	OR482983		OR483007	OR483032
<i>Synalpheus bituberculatus</i>	1291	Singapore	OUMNH.ZC.2014-11-190	OR482982		OR483006	OR483031
<i>Synalpheus bocas</i>	16	Jamaica	JAM08-7402	KJ595041	KJ625107	HQ435426	KJ595249
<i>Synalpheus bousfieldi</i>	483	Belize	CBC09-3605	KJ595042	KJ625073		KJ595250
<i>Synalpheus brevifrons</i>	481	Belize	CBC09-2704	KJ625034	KJ625072	HQ435435	KJ595251
<i>Synalpheus brooksi</i>	476	Panama	P09-3102	KJ595049	KJ625068	HQ435437	KJ595253
<i>Synalpheus brooksi</i>	479	Panama	P09-4911	KJ625035	KJ625070	HQ435438	KJ595252
<i>Synalpheus carpenteri</i>	421	Jamaica	JAM08-3008	KJ595052		HQ435439	KU682628
<i>Synalpheus cayoneptunus</i>	910	Florida	VIMS 13FK2901, 2903	KM204166		KM204180	KU682629
<i>Synalpheus chacei</i>	457	Belize	CBC09-501	KJ595059	KJ625062	HQ435440	KJ595265
<i>Synalpheus chaki</i>	2014_1228	Martinique	MNHN-IU-2014-1228	MZ323456		MZ329362	
<i>Synalpheus charon</i> A	565	Egypt	OUMNH.ZC.2014-02-022	KJ595060	KJ625128	KJ595191	KJ595266
<i>Synalpheus charon</i> C	566	Taiwan	FLMNH-UF 19914	KJ595064		KJ595192	KJ595269
<i>Synalpheus corallinus</i>	826	Jamaica	JAM12-9902	KU980212		HQ435441	KU682630
<i>Synalpheus corbariae</i>	2014-1238	Martinique	MNHN-IU-2014-1238	MZ323450		MZ329367	
<i>Synalpheus coutierei</i> B	2164	French Poly-nesia	FLMNH-UF 16178	OR482984		OR483008	OR483033
<i>Synalpheus dardeau</i>	461	Belize	CBC09-3105	KJ625036	KJ625110	HQ435442	KJ595271
<i>Synalpheus dominicensis</i>	482	Belize	CBC09-2906	KJ477702	KJ494390	KJ477695	KU682632
<i>Synalpheus duffyi</i>	26	Jamaica	JAM08-7403 (26), VIMS 08JAM7401-2	KJ595078		HQ435444	KU682633
<i>Synalpheus elizabethae</i>	944, 137	Panama	P08-12504 (137)	KU980213		HQ435446	KU682634
<i>Synalpheus flidigitus</i>	470	Belize	CBC09-7603	KJ595079	KJ625066	HQ435447	KJ595275
<i>Synalpheus fossor</i>	570	Thailand	OUMNH.ZC.2011-03-096	KJ595080		KJ595199	KJ595276
<i>Synalpheus goodei</i>	464	Belize	CBC09-5404	KJ477698	KJ625065	HQ435448	KJ595279
<i>Synalpheus guerini</i> A	527, 541	Florida	FLMNH-UF 9334	KJ595082		KJ595200	KJ595282
<i>Synalpheus gustavi</i> n. sp.	707	Madagascar	FLMNH-UF 14255-1				KJ494394
<i>Synalpheus gustavi</i> n. sp.	2172	Madagascar	FLMNH-UF 14231	OR482985		OR483010	
<i>Synalpheus gustavi</i> n. sp.	2173	Madagascar	FLMNH-UF 14259	OR482986		OR483011	
<i>Synalpheus gustavi</i> n. sp.	2175	Madagascar	FLMNH-UF 14255	OR482987		OR483012	
<i>Synalpheus gustavi</i> n. sp.	2184	Madagascar	FLMNH-UF 14257			OR483009	
<i>Synalpheus gustavi</i> n. sp.	2180	Madagascar	FLMNH-UF 14236			OR483013	
<i>Synalpheus hastilicrassus</i> A	Syn2119	Queensland	FLMNH-UF 24728	OR482988		OR483015	
<i>Synalpheus hastilicrassus</i> A	678	Queensland	FLMNH-UF 17136	KJ625055	KJ625098		KJ595284
<i>Synalpheus hastilicrassus</i> B	596	Queensland	FLMNH-UF 18204	KJ595089	KJ625143	KJ595206	KJ595287
<i>Synalpheus hastilicrassus</i> C	Syn2111	Hong Kong	FLMNH-UF 54147	OR482989	KJ625134	OR483016	KJ595288
<i>Synalpheus hastilicrassus</i> _D	Syn2109	Guam	FLMNH-UF 37332	OR482990		OR483017	
<i>Synalpheus hastilicrassus</i> _F	Syn2118	Queensland	FLMNH-UF 18256	KJ625061	KJ625134	OR483014	KJ595288
<i>Synalpheus hemphilli</i>	571	Florida	FLMNH-UF 19640	KJ595092		KJ595208	KJ595290
<i>Synalpheus herricki</i>	157	Curacao	CU08-3202	KJ595095		HQ435449	KU682635
<i>Synalpheus hoetjesi</i>	202	Curacao	CU08-2901	KJ625037	KJ625076	HQ435452	KJ595293
<i>Synalpheus idios</i>	474	Belize	CBC09-8803	KJ625038	KJ625115	HQ435455	
<i>Synalpheus iocasta</i>	1284	Singapore	OUMNH.ZC.2014-11-219	OR482991		OR483018	OR483034
<i>Synalpheus irie</i>	38, 493	Jamaica	JAM08-3601	KJ595106	KJ625117	HQ435457	KJ595294
<i>Synalpheus kensleyi</i>	504	Panama	P07-1204	KJ625039	KJ625119	HQ435458	KJ595295

Species	Genetics#	Locality	Collection#	COI	PEPCK	16S	18S
<i>Synalpheus kuadramanus</i>	843	Jamaica	VIMS 12JAM9501	KU980214		MZ329378	KU682637
<i>Synalpheus lani</i>	599	Panama	OUMNH.ZC.2014-04-030	KJ595107	KJ625145	KJ595210	KJ595296
<i>Synalpheus</i> aff. <i>longicarpus</i>	488	Panama	P09-9101	KJ595025	KJ625075	HQ435459	KJ595240
<i>Synalpheus macdonaldi</i>	2014_1227	Martinique	MNHN-IU-2014-1227	MZ323448		MZ329364	
<i>Synalpheus mcclendoni</i>	237	Barbados	BR08-1413	KJ595109		HQ435462	KU682639
<i>Synalpheus microneptunus</i>	247	Barbados	BR08-6001	KJ595110	KJ625108	HQ435463	KU682640
<i>Synalpheus</i> aff. <i>sanctithomae</i>	471	Belize	CBC09-7804	KJ625043	KJ625114	HQ435464	KJ595243
<i>Synalpheus</i> aff. <i>sanctithomae</i>	486	Belize	CBC09-8701	KJ595029	KJ625074	HQ435465	KJ595244
<i>Synalpheus neomeris</i> _A	1133	Saudi Arabia	FLMNH-UF 36916	OR482992		OR483019	OR483035
<i>Synalpheus neomeris</i> _A	573	Madagascar	FLMNH-UF 12540	KJ595056		KJ595188	KJ595260
<i>Synalpheus neptunus</i> A	560	Queensland	FLMNH-UF 17566	KJ595119	KJ625126	KJ595217	
<i>Synalpheus neptunus</i> B	1119	Queensland	FLMNH-UF 23841	OR482994		OR483021	
<i>Synalpheus</i> cf. <i>neptunus germanus</i>	1117	Western Australia	FLMNH-UF 28020-7	OR482993		OR483020	OR483036
<i>Synalpheus nobilii</i>	576	Panama	OUMNH.ZC.2013-03-009	KJ595122	KJ625132	KJ595219	KJ595303
<i>Synalpheus obtusifrons</i>	465	Belize	CBC09-6303	KJ477703	KJ494389	HQ435466	KJ494396
<i>Synalpheus pandionis</i>	472	Belize	CBC09-8403	KJ595126	KJ625067	HQ435468	KJ595305
<i>Synalpheus parfaii</i>	609	Sao Tome	MNHN-IU-2010-4150	KJ595127		KJ595223	
<i>Synalpheus pectiniger</i>	500	Jamaica	JAM08--8801	KJ595129	KJ625118	HQ435470	KJ595307
<i>Synalpheus peruvianus</i>	601	Panama	OUMNH.ZC.2013-03-056	KJ595132	KJ625147	KJ595224	KJ595310
<i>Synalpheus plumosetosus</i>	231	Jamaica	JAM08-2704	KU980220		HQ435471	
<i>Synalpheus rathbunae</i>	941	Panama	P08--3501-2	KU980221		AY344767	KU682644
<i>Synalpheus regalis</i>	469	Belize	CBC09-7002	KJ625042	KJ625113	HQ435474	
<i>Synalpheus ruetzleri</i>	466	Belize	CBC09-6201	KJ595136	KJ625112	HQ435475	KJ595313
<i>Synalpheus sanctithomae</i>	235	Barbados	BR08-1201	KJ595139		AY344768	KU682647
<i>Synalpheus sanlucasi</i>	654	Panama	OUMNH.ZC.2013-03-015	KJ625049	KJ625089	KJ595225	KJ595317
<i>Synalpheus scaphoceris</i>	683	Panama	MNHN-IU-2010-4152	KJ625058		KJ595226	KJ595318
<i>Synalpheus sladeni</i>	1137	Madagascar	FLMNH-UF 14474	OR482995		OR483022	OR483037
<i>Synalpheus somalia</i> aff.	2186	Saudi Arabia	FLMNH-UF 36964	OR482996		OR483023	OR483038
<i>Synalpheus spinifrons</i>	611	Chile	AA--07-317	KJ595145		KJ595228	KJ595321
<i>Synalpheus sponjy</i> n. sp.	2178	Madagascar	FLMNH-UF 14228	OR482998		OR483025	OR483039
<i>Synalpheus sponjy</i> n. sp.	2176	Madagascar	FLMNH-UF 14256	OR482997		OR483024	
<i>Synalpheus sponjy</i> n. sp.	2179	Madagascar	FLMNH-UF 14316	OR482999		OR483026	
<i>Synalpheus stimpsonii</i> _A	553	Queensland	FLMNH-UF 16622	KJ595147		KJ595229	KJ595323
<i>Synalpheus stimpsonii</i> _B	554	Queensland	FLMNH-UF 16982	KJ595148		KJ595230	KJ595325
<i>Synalpheus stimpsonii</i> _C	1109	Queensland	FLMNH-UF 16788	OR483000		OR483027	KJ595326
<i>Synalpheus streptodactylus</i> A	584	Madagascar	FLMNH-UF 13933	KJ595151		KJ595234	KJ595327
<i>Synalpheus thai</i>	1281	Singapore	OUMNH.ZC.2014-11-263	OR483001		OR483028	OR483040
<i>Synalpheus theano</i>	1286	Singapore	OUMNH.ZC.2014-11-266	OR483002		OR483029	OR483041
<i>Synalpheus theano</i>	1285	Singapore	OUMNH.ZC.2014-11-264	OR483003			
<i>Synalpheus theano</i>	2183	Western Australia	FLMNH-UF 21825	OR483004			
<i>Synalpheus thele</i>	113	Jamaica	JAM08-8924	KJ595156	KJ625106		KJ595335
<i>Synalpheus ul</i>	253	Barbados	BR08-8703	KJ625044	KJ625109	HQ435482	KU682648
<i>Synalpheus williamsi</i>	462	Belize	CBC09-5102	KU980224	KJ625064	HQ435484	KJ595338
<i>Synalpheus yano</i>	484	Belize	CBC09-3802	KJ595161	KJ625116	HQ435485	KJ595339
<i>Alpheus percyi</i> (outgroup)	0		FLMNH-UF 12372	KJ477697	KJ494392	KJ477694	KJ494395

2.2. DNA extraction, amplification, and sequencing

Tissue from the gills, eggs, and/or pleopods 3-5 were used for genomic DNA extraction using the QIAGEN DNeasy Blood and Tissue kit, following standard manufacturer protocols. We amplified the COI and 16S loci using PCR and thermocycler conditions described in Ashrafi and Hultgren (2022), and 18S described in Hult-

gren et al. (2014). PEPCK sequences were acquired from GenBank from a previous study (Hultgren et al. 2014). For COI, we used the primer pairs jgLCO/jgHCO (Geller et al. 2013), or polyLCO/polyHCO from Brasier et al. (2016). For 16S and 18S, we used the primers, PCR conditions, and thermocycler settings described in Hultgren et al. (2014). PCR products were cleaned up using EXOSAP and sequenced for forward and reverse sequences at MCLab (South San Francisco, CA).

2.3. Tree Construction

Forward and reverse sequences were cleaned, aligned, and translated to amino acids to check for stop codons using Sequencher (Gene Codes, Ann Arbor, MI, USA) and MEGA v. 11 (Stecher et al. 2020). All new sequences were aligned with the existing sequences for the four loci (PEPCK, 18S, 16S, and COI) from Hultgren et al. (2014) and Chak et al. (2021) using MUSCLE (Edgar et al. 2004) implemented in MEGA X (Stecher et al. 2020). For the 18S alignment, we used GBLOCKS v. 0.91.1 (Castresana, 2000; Talavera and Castresana 2007) to exclude ambiguous areas of the alignment using relaxed criteria (allowed gap positions = with half, minimum length of a block = 5), yielding an alignment of 253 bp. For COI, we trimmed the alignment to ~660 bp at the 5' end of the COI gene (corresponding to the standard barcoding region). For the final tree, we included all taxa with at least three out of the four loci sequenced (all taxa were sequenced for COI), to optimize taxon sampling. We used JModelTest ver. 2.1.10 (Darriba et al. 2012) to get the best model of nucleotide substitution using AIC, and implemented these models in a concatenated Bayesian analysis using MrBayes v.3.27 (Nylander et al. 2004; Ronquist et al. 2012) run on the CIPRES server (Miller et al. 2010). The Bayesian tree was run for 1×10⁸ generations with four chains, and we discarded the first 25% of the samples as burn-in. As we generated many additional sequences of COI for new specimens that were not included in the consensus tree, we also ran a single-gene Bayesian tree using COI data for 3×10⁸ generations (again discarding the first 25% of the samples as burn-in).

2.4. Abbreviations

CL – Carapace length; FLMNH – Florida Museum of Natural History, Gainesville, Florida.

3. Results

3.1. Phylogenetic trees

The consensus phylogenetic tree constructed in this study (Fig. 1) is based on a concatenation of 16S, COI, 18S, and PEPCK sequences. We incorporated major clades or representative species from previous studies (Hultgren et al., 2014; Chak et al. 2021) and included new species as well as morphologically similar species to gain a better understanding of the phylogenetic relationships among eusocial species in the Indo-West Pacific region. However, due to the unavailability of fresh material, we were unable to include the other known eusocial species from the Western Indian Ocean, *S. crosnieri* Banner and Banner, 1983, and *S. paradoxus* Banner and Banner, 1981, in the present analysis. Additionally, we added *S. cf. neptunus germanus*, a potential eusocial species from the Eastern

Indian and western Pacific oceans, to the phylogenetic analysis.

The results of the phylogenetic study using Bayesian inference (Fig. 1) indicate that eusociality has independently evolved in each eusocial species from the Indo-Pacific region included in this analysis. Eusociality arose once in the *S. neptunus* clade, which comprises *S. neptunus neptunus* (Dana 1852), *S. neptunus germanus* Banner and Banner, 1975, and *S. theano* De Man, 1910. It also arose once in *S. sponjy* **sp. nov.**, which is positioned as a sister to a clade consisting of *S. sladeni* Coutière, 1908, *S. thai* Banner and Banner, 1966, and *S. aff. somalia* Banner and Banner, 1979. Furthermore, eusociality emerged in *S. gustavi* **sp. nov.**, which is not closely related to other species in the tree. Notably, the latter species holds an isolated position relative to all other *Synalpheus* species in the Indo-West Pacific.

The previously recognized *S. gambarelloides* group is no longer a monophyletic clade (possibly due to the lack of morphological characters). Several of its members are now positioned outside the traditional *S. gambarelloides* clade. *Synalpheus rathbunae* Coutière, 1909, group; *S. paranepetunus* Coutière, 1909, group; *S. herricki* Coutière, 1909; *S. pectiniger* Coutière, 1907; *S. androsi* Coutière, 1909; *S. kensleyi* (Ríos and Duffy 2007); *S. parfaiti* (Coutière, 1898); *S. agelas* Pequegnat and Heard, 1979; and *S. mcclendoni* Coutière, 1910 are the species situated outside the main body of the *S. gambarelloides* clade.

In the current phylogenetic study, we identify seven distinct lineages of eusociality: the entire *S. rathbunae* clade; appearing multiple times within the *S. paranepetunus* clade; within *S. brooksi* Coutière, 1909, species complex; as well as involving *S. chacei*; *S. gustavi* **sp. nov.**; *S. sponjy* **sp. nov.**; and at least two occurrences within the *S. neptunus* species complex. While *S. crosnieri* is not encompassed within the phylogenetic analysis, it is undoubtedly closely related to *S. sponjy* **sp. nov.** owing to numerous morphological similarities between the two species. Moreover, it is noteworthy that *S. paradoxus* might potentially introduce a new lineage of eusociality within the genus.

The COI Bayesian gene tree (Fig. S1) indicated that multiple sequenced specimens of the two new species are reciprocally monophyletic, with Bayesian posterior probabilities = 1.

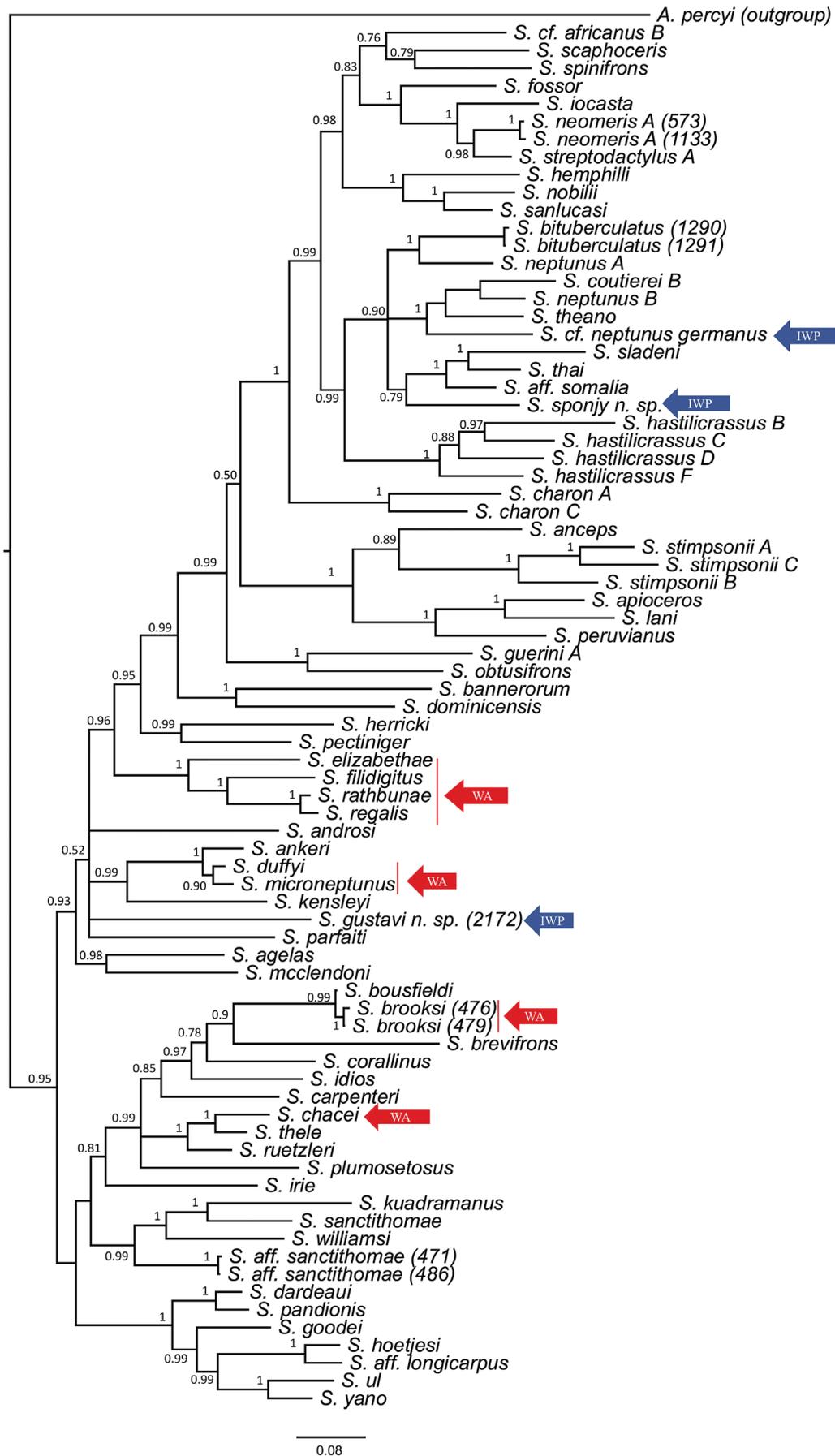


Figure 1. Phylogenetic tree constructed for the members of the genus *Synalpheus* using the Bayesian Inference approach based on a concatenation of four genes: 16S, COI, PEPCK, and 18S. Bayesian posterior probabilities are provided above each branch. Taxa are shown as *Synalpheus* species plus unique identifier (see supplementary table S1). Eusocial species are indicated with an arrow (red, WA= West Atlantic; blue, IWP = Indo-West Pacific).

3.2. Taxonomy

Infraorder Caridea Dana, 1852

Family Alpheidae Rafinesque, 1815

Genus *Synalpheus* Spence Bate, 1888

3.2.1. *Synalpheus sponjy* sp. nov.

<https://zoobank.org/220DDF9F-08CD-4510-93DC-F4E0214F5761>

Figures 2–4

Etymology. The new species derives its name from the Malagasy word ‘sponjy’, which translates to ‘sponge’. The term is used as a noun in opposition.

Material examined. *Holotype:* MADAGASCAR • 1 male (CL 3.6 mm); Nosy Be; 13°25'9.7"S, 48°15'37.94"E; 17 May 2008; Bakary G, Bruggermann H, Michonneau F, Paulay G, Werner T leg.; 6–9 m, from cryptic sponge; FLMNH-UF-14409. — *Paratypes:* MADAGASCAR • 19 males (CL ranging from 1.99 to 3.6 mm); Nosy Vorona; 15 May 2008; Paulay G leg.; 4 m, from cryptic sponge; FLMNH-UF-14256 • 2 males (CL 3.1–3.2 mm); same data as for preceding; FLMNH-UF-14228 • 1 male (CL 3.35 mm); same data as for preceding; Anker A, Bakary G, Boissin E, Bruggemann H, Horeau T, Michonneau F, Paulay G, Werner T leg.; FLMNH-UF-14313 • 1 male (CL 3.23 mm); same data as for preceding; FLMNH-UF-14316.

Description. Small-sized species of *Synalpheus*. Carapace (Fig. 2A, B) glabrous, unarmed; frontal margin between rostrum and orbital teeth relatively deep, U-shaped, with moderately developed rostrum; rostrum triangular in dorsal view, subacute at tip, about twice as long as wide, reaching to about proximal third of visible part of first antennular article, slightly shorter than orbital teeth, orbitorostral process present; orbital teeth relatively stout, subtriangular, distally subacute, as long as wide, about three times as broad as rostrum at base; pterygostomial angle bluntly protruding; cardiac notch deep.

Male pleon (Fig. 2C) with first pleuron bearing small ventrally-pointing hook-shaped projection posteroventrally; third pleuron rounded anteroventrally and angled posteroventrally; fourth pleuron rounded anteroventrally and subrounded posteroventrally; fifth pleuron rounded antero- and posteroventrally; sixth pleuron with blunt projection on posterolateral margin.

Telson (Fig. 2D) subrectangular, tapering distally, slightly shorter than endopod, flat, without depression on mid-line; dorsal margin with two pairs of relatively strong spiniform setae located at 0.3 and 0.6 telson length, respectively; posterolateral angle with no projection; posterior margin broad, slightly convex, furnished with about 10 setae, with two pairs of spiniform setae, mesial spiniform setae slightly longer than lateral ones.

Antennula (Fig. 2A, B) with peduncle relatively stout; first antennular article almost twice as long as broad; stylocerite relatively short, subacute, falling short of distal

margin of first antennular article; second antennular article slightly shorter than first one, slightly longer than broad; third antennular article slightly longer than second one, subequal to first one, slightly longer than broad; lateral flagellum biramous, fused portion consisting of five subdivisions, secondary ramus with three subdivisions, aesthetascs extending from third subdivision of fused portion to end of secondary ramus.

Antenna (Fig. 2A, B) with inferior tooth of basicerite subacute, slightly overreaching first antennular article, superior tooth small, subacute; scaphocerite relatively slender, lateral tooth overreaching end of second antennular article, lateral margin straight, blade fully absent; carapocerite somewhat slender, extending beyond antennular peduncle.

Mouthparts not dissected. Third maxilliped (Fig. 2E) pediform; lateral plate with small acute hooked-shaped projection pointing laterally; antepenultimate article about 4 times as long as widest part, mesial margin furnished with setae; penultimate article short, about a sixth of antepenultimate length; ultimate article relatively stout, about 0.6 times as long as antepenultimate article, slightly tapering distally, with crown of six spiniform setae at tip; exopod slender, reaching to about 0.8 of antepenultimate article length.

Major cheliped (Fig. 3A–D) with coxa bearing small spiniform seta on dorsodistal margin; basis and ischium short; merus robust, dorsal margin convex, about twice as long as broadest part, dorsodistal margin with blunt projection; carpus short, cup-shaped; palm swollen, ovoid, about 1.5 times as long as wide, dorsodistal margin with subrounded, prominent tubercle; fingers 0.4 times as long as palm, pollex subequal to dactylus. Minor cheliped (Fig. 3E, F) with coxa bearing small spiniform seta on dorsodistal margin; basis and ischium short; merus relatively robust, ventral margin concave, about 3 times as long as broadest part; carpus vase-shaped; palm ovoid, nearly 0.7 times as long as merus, about 0.7 times as long as wide; fingers subequal, about 0.8 times as long as palm; pollex with several tufts of setae situated on lateral side, simple at tip; dactylus bearing densely-situated tufts of distally-curved setae (known as gambarelloides setae), simple at tip.

Second pereiopod (Fig. 4A) with coxa bearing small spiniform seta dorso-distally; basis short; ischium relatively robust, approximately 3.5 times as long as broad; merus relatively robust, 3.5 times as long as broad, about 1.3 times as long as ischium; carpus slightly longer than merus, composed of five articles, proximal article longest, as long as sum of three medial articles, second to fourth articles subequal, distal article twice as long as fourth one; palm subequal to fingers.

Third pereiopod (Fig. 4B, C) robust, coxa with small spiniform setae on dorsodistal margin; ischium vase-shaped, 1.5 times as long as broadest part; merus robust, about 3 times as long as broad, about 2.8 times as long as ischium, distal half of ventral margin concave, mesial margin of concavity furnished with thick setae and lateral margin widened; carpus slightly widening distally, slightly longer than ischium, with spiniform seta on distoven-

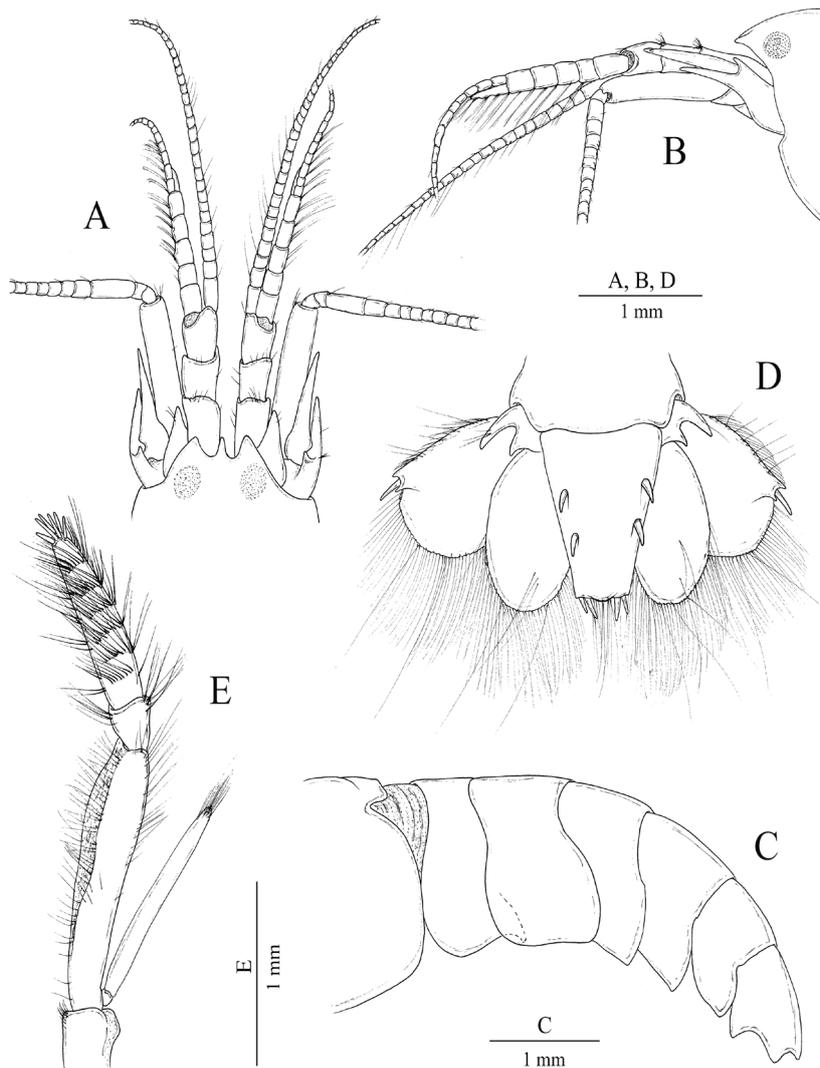


Figure 2. *Synalpheus sponjy* sp. nov., holotype (FLMNH-UF-14409). **A** anterior region, dorsal view **B** same, lateral view, **C** pleon, **D** telson and uropods, **E** third maxilliped.

tral margin; propodus with five spiniform setae on ventral margin in addition to distal pair, about 1.7 times as long as carpus; dactylus biunguiculate, inferior unguis slightly wider at base than superior one, latter one slightly longer than former. Fourth pereiopod (Fig. 4D) generally similar to third pereiopod, less robust; coxa lacking spiniform setae on dorso-distal margin; merus concavity less prominent and mesial margin lacking thick setae. Fifth pereiopod (Fig. 4E) generally similar to third pereiopod, less robust; merus without concavity; carpus about 0.7 times as long as merus, without spiniform setae on distoventral margin; propodus subequal to merus, bearing four transverse rows of microserrulate setae on distal half.

Uropods (Fig. 2D) with protopods bearing two subacute teeth; exopod with small distolateral tooth; diaeresis inconspicuous except for lateral margin terminating to small tooth; distolateral spiniform setae next to diaeresis stout, not overreaching posterior margin of exopod.

Type locality. North of Madagascar: Nosy Be and Nosy Vorona.

Ecology. All the specimens were collected from cryptic sponges situated among dead coral and coral rubbles.

Remarks. The new species exhibits similarities to several rare species found in the Indo-West Pacific region, characterized by a dense brush of setae on the dactylus of the minor cheliped, known as gambarelloides setae. These species include *S. sladeni* Coutière, 1908; *S. spongicola* Banner and Banner, 1981; *S. crosnieri* Banner and Banner, 1983; and *S. gambarelloides* (Nardo, 1847) [as reported in Banner and Banner (1983)]. Originally described from Cargados Carajos Shoals (Mauritius) and reported in the Gulf of Aqaba [Banner and Banner (1981)], *S. sladeni*, each time based on a single specimen, can be distinguished from the new species by several notable characteristics: slender orbital teeth, presence of a scaphocerite blade, less prominent inferior tooth and more prominent superior tooth of the basicerite, highly robust exopods of the second and third maxillipeds, a subacute tooth on the dorso-distal margin of the major chela palm, and complete presence of diaeresis.

According to Banner and Banner (1983), 45 males and 6 females, suggesting a potential communal lifestyle for the species, from Tuléar, Madagascar were reported and identified as *S. gambarelloides*, (for further details, see Banner and Banner 1983: page 97). However, no descriptions or illustrations were provided for these specimens,

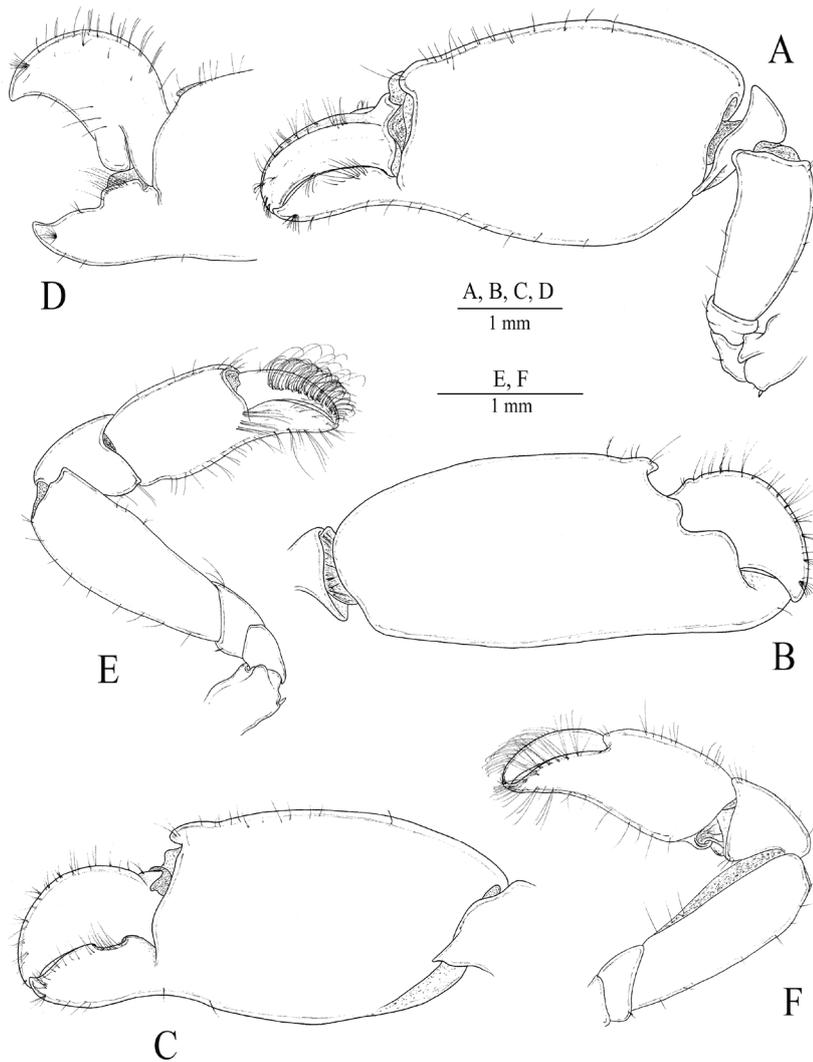


Figure 3. *Synalpheus sponjy* **sp. nov.**, holotype (FLMNH-UF-14409). **A** major cheliped, **B** same, chela, **C** lateral view same, **D** mesial view same, **E** fingers minor cheliped, **F** mesial view same, lateral view.

except for one distinguishing characteristic of *S. gambarelloides*, namely the absence of an orbitorostral process. This particular trait can be utilized to differentiate the new species from *S. gambarelloides*.

Synalpheus sponjy **sp. nov.** is closely related to the other two species, *S. crosnieri* and *S. spongicola*, both of which have not been reported since their original descriptions. The new species can be distinguished from *S. crosnieri* (found northwest of Madagascar) by the overall shape of the major cheliped fingers. In *S. sponjy* **sp. nov.**, the major cheliped is normal and straight, whereas in *S. crosnieri*, it appears twisted when viewed dorsally. Additionally, the shape of the meri in the third and fourth pereopods sets the new species apart. In the new species, these meri are concave on the distal half, and the third pereopod merus bears a row of stiff setae along the mesial margin of the concavity. However, caution should be exercised when using the latter characteristic as the drawings provided by Banner and Banner (1983) are schematic, and the description lacks precise information regarding the exact shape of the meri in the third and fourth pereopods.

One of the authors (HA) had the opportunity to examine the type series of *S. spongicola* deposited in the Naturalis Biodiversity Center, Leiden, Netherlands. As mentioned by Banner and Banner (1981), *S. spongicola*

is a relatively large species of *Synalpheus*. In addition to differences in size, *S. sponjy* **sp. nov.** can be distinguished from *S. spongicola* based on the following characters: 1) the stylocerite falls short of reaching the end of the first antennular article in the new species, whereas in *S. spongicola*, it extends to the end; 2) the blade of the scaphocerite is absent in the new species, while it is complete in *S. spongicola*; 3) the palm of the major cheliped terminates in an obtuse, robust tubercle that points forward on the dorso-distal margin in *S. sponjy* **sp. nov.**, whereas in *S. spongicola*, it ends in an upturned subacute tooth; 4) the last three pereopods are more robust in the new species, and the meri of the third and fourth pereopods are ventrally concave (lacking concavity in *S. spongicola*); and 5) in the new species, the diaeresis is confined to the lateral tooth, whereas in *S. spongicola*, the diaeresis is complete. Unfortunately, as these types were very old (date), they could not be included in the phylogenetic tree. Limited information is available regarding the ecology of *S. spongicola*, as it has been recorded only once with 9 females and 1 male from a single collection, suggesting a potential communal lifestyle for the species.

In the phylogenetic tree (Fig. 1), *S. sponjy* **sp. nov.** forms a clade with *S. aff. somalia* (Banner and Banner, 1979) and *S. thai* (Banner and Banner, 1966), along with

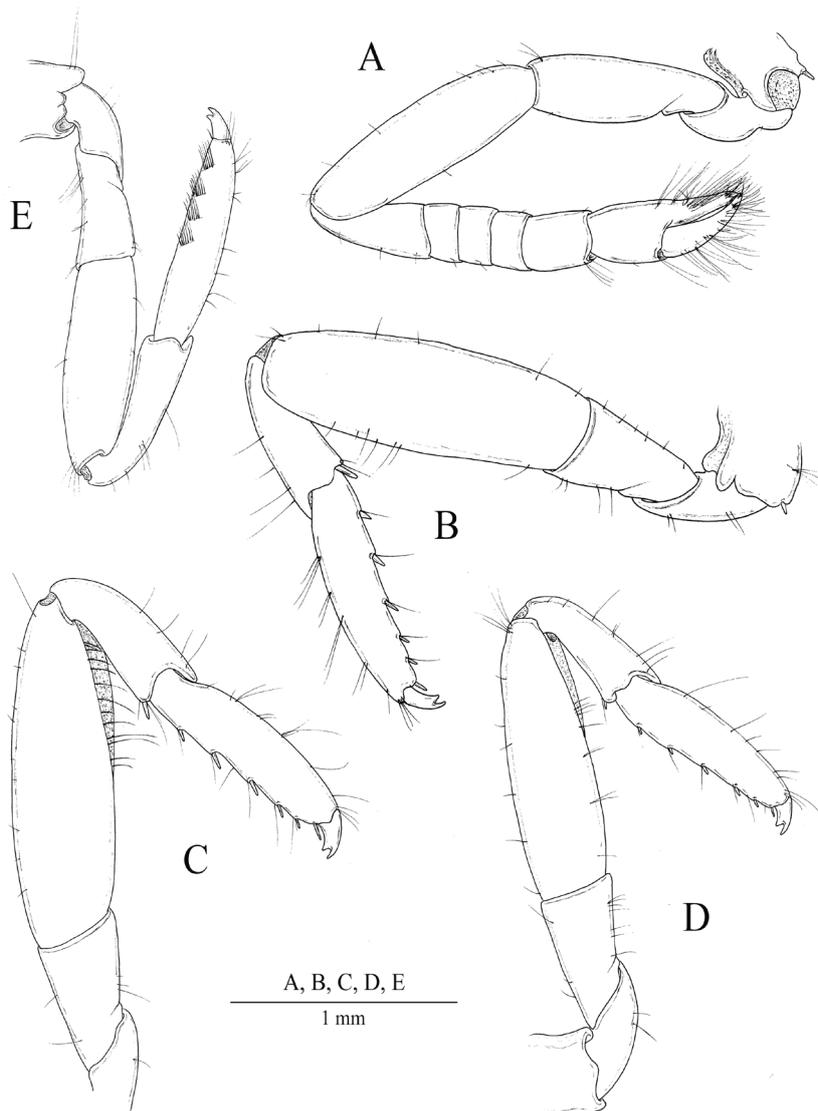


Figure 4. *Synalpheus sponjy* sp. nov., holotype (FLMNH-UF-14409). **A** second pereopod, **B** third pereopod, **C** lateral view same, mesial view, **D** fourth pereopod, mesial view, **E** fifth pereopod, lateral view.

S. sladeni. The new species can be easily distinguished from both *S. somalia* and *S. thai* by the densely-located (gambarelloides) setae on the dactylus of the minor cheliped. Additionally, *S. thai* stands out within the entire genus due to its distinctive shape of the telson (see Banner and Banner 1966: fig. 19L). *Synalpheus somalia* can be further differentiated from the new species by the dactylus of the major cheliped being much longer than the pollex (Banner and Banner 1979: fig. 3c, e).

3.2.2. *Synalpheus gustavi* sp. nov.

<https://zoobank.org/F4A283FB-9423-4698-BAC8-F71D548E0F74>

Figures 5–7

Synalpheus aff. *brevifrons* Hultgren, Hurt and Anker, 2014; Ashrafi and Hultgren, 2022.

Etymology. The new species is named after Gustav Paulay (FLMNH), who collected the majority of the speci-

mens that contributed to the present study, and in recognition of his generous help and warm hospitality during the authors' visit to the FLMNH collection.

Material examined. **Holotype:** MADAGASCAR • 1 male (CL 3.4 mm); Nosy Vorona; 15 May 2008; Paulay G leg.; 4m, in cryptic sponge; FLMNH-UF-71394. — **Allotype:** MADAGASCAR • 1 female (CL 3.6 mm); same data as for holotype; FLMNH-UF-71395. — **Paratypes:** MADAGASCAR • 15 males (CL ranging from 1.99 to 2.98 mm); from the allotype colony; FLMNH-UF-14259 • 4 males (CL ranging from 1.73 to 2.98 mm); from the holotype colony; FLMNH UF-14255 • 19 males and 0 females (CL ranging from 1.37 to 3.35 mm); same data as for holotype; FLMNH-UF-14257 • 1 female and 3 males (CL ranging from 1.73 to 2.85 mm); same data as for holotype; FLMNH-UF-14231 • 1 female and 3 males (CL ranging from 2.86 to 3.35 mm); same data as for holotype; FLMNH-UF-14230 • 1 male (CL 2.23 mm); Nosy Vorona; 15 May 2008; Anker A leg.; cryptic sponge; FLMNH-UF-14915 • 2 males (CL 2.85–2.98 mm); same data as for holotype; FLMNH-UF-14229 • 5 males (CL ranging from 2.36–3.48); same data as for holotype; FLMNH-UF-14236.

Description. Small-sized species of *Synalpheus*. Carapace (Fig. 5A, B) glabrous, smooth; frontal margin

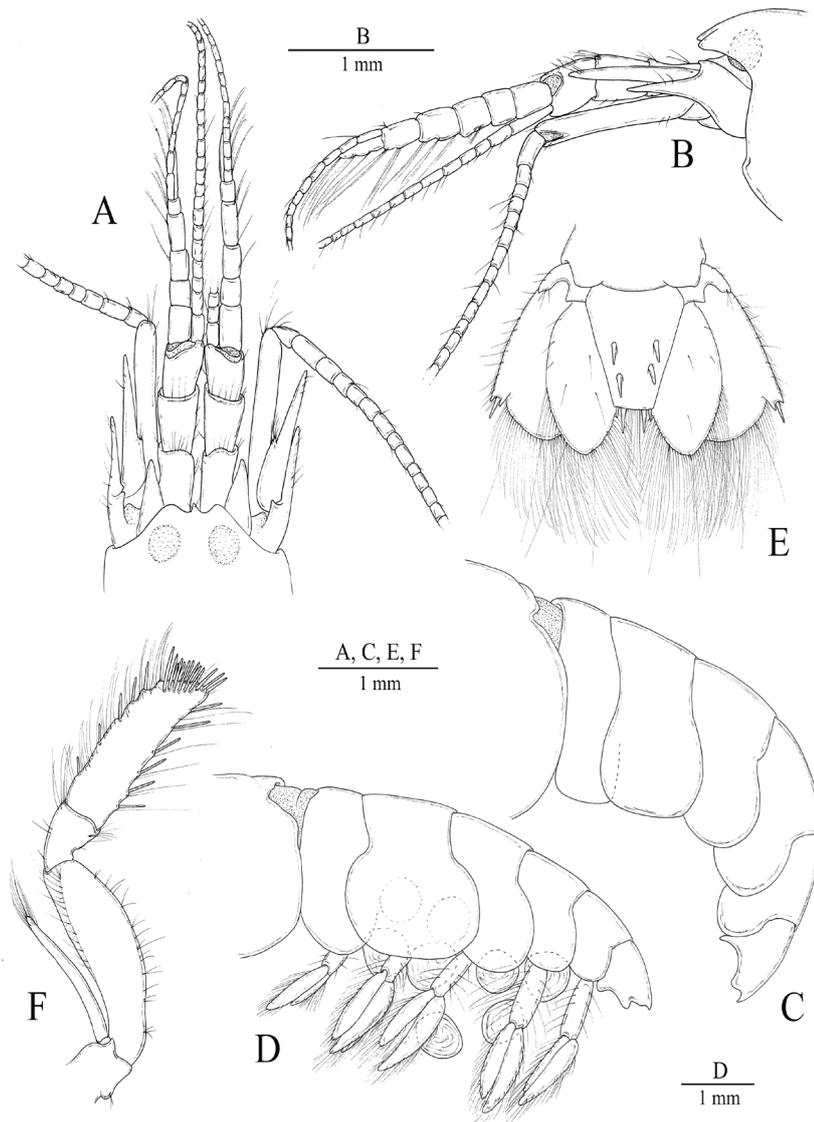


Figure 5. *Synalpheus gustavi* sp. nov., holotype (FLMNH-UF-71394). **A** anterior region, **B** dorsal view same, **C** lateral view pleon, **D** same, allotype, **E** telson and uropods, **F** third maxilliped.

between rostrum and orbital teeth shallow, broadly U-shaped, with very small rostrum; rostrum triangular in dorsal view, subacute at tip, approximately as long as wide, slightly surpassing orbital teeth, orbitorostral process present; orbital teeth shallow, broad, distally subrounded, about four times as broad as long, about three times as broad as rostrum at base; pterygostomial angle bluntly protruding; cardiac notch relatively deep.

Pleon only showing sexual dimorphism in first pleonite. Male pleon (Fig. 5C) with first pleuron angulate or with tiny projection posteroventrally; third to fifth pleuron rounded anteroventrally and posteroventrally. Female pleon (Fig. 5D) with first pleuron rounded posteroventrally.

Telson (Fig. 5E) subrectangular, tapering posteriorly, remarkably shorter than endopod, approximately as long as broadest part, dorsal margin with two pairs of relatively strong spiniform setae at 0.5 and 0.8 telson length (right pairs in holotype), respectively, without depression on mid-line; distolateral angle with no projection; posterior margin broad, straight, furnished with 8 setae, with two pairs of spiniform setae, mesial spiniform setae about twice as long as lateral ones and subequal to dorsal spiniform setae.

Antennula (Fig. 5A, B) with peduncle relatively stout; first antennular article about 1.7 times as long as broad; stylocerite relatively short, subacute at tip, falling short of reaching distal margin of first antennular article; second antennular article subequal to first one, slightly longer than broad; third antennular article shortest, about 0.8 times as long as first antennular article, slightly longer than broad; lateral flagellum biramous, fused portion consisting of five subdivisions, aesthetascs extending from third subdivision of fused portion to end of secondary ramus.

Antenna (Fig. 5A, B) with inferior tooth of basicerite subacute, reaching to about middle of second antennular article, superior tooth small, subacute; scaphocerite relatively slender, lateral tooth reaching to midlength of third antennular article, lateral margin straight, blade remarkably reduced; carpopocerite slender, extending beyond antennular peduncle.

Mouthparts not dissected. Third maxilliped (Fig. 5F) pediform; lateral plate with small hooked-shaped projection pointing laterally; antepenultimate article about 3 times as long as widest part; penultimate article short, about a fourth of antepenultimate article length; ultimate

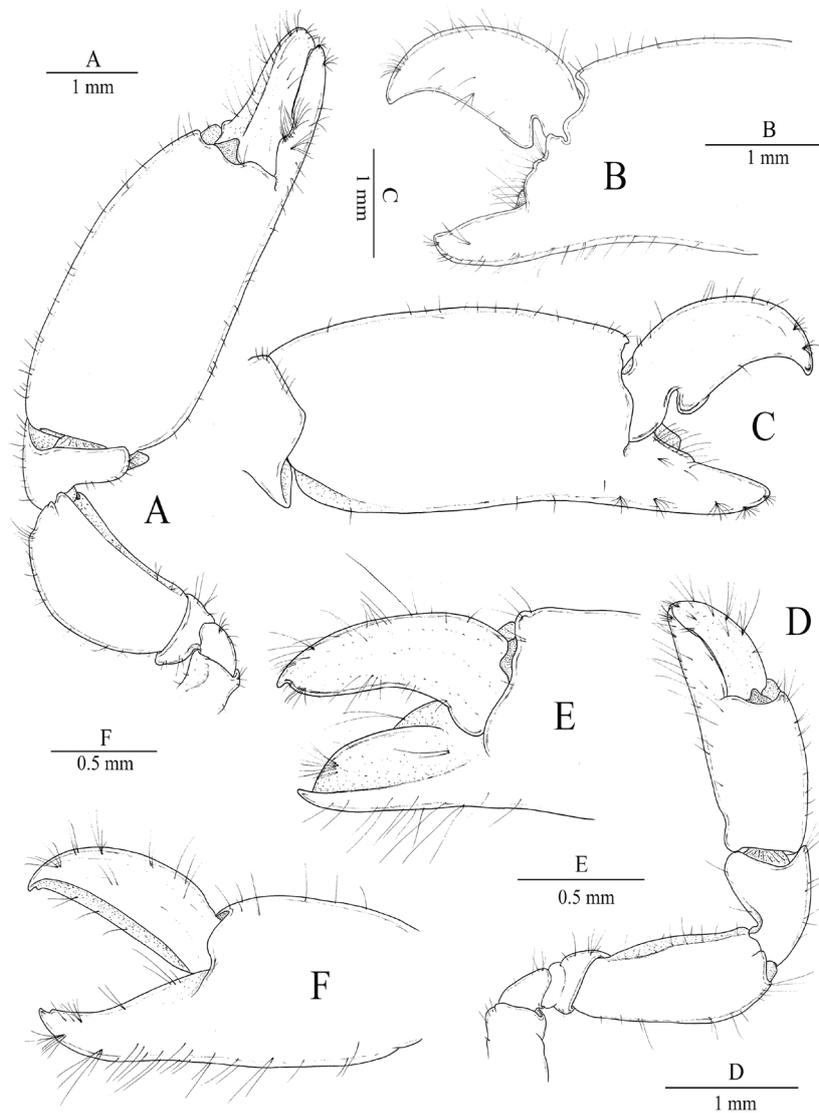


Figure 6. *Synalpheus gustavi* sp. nov., holotype (FLMNH-UF-71394). **A** major cheliped, **B** same, chela, **C** lateral view same, **D** mesial view minor cheliped, **E** same, fingers, dorsomesial view, **F** same, ventromesial view.

article very stout, not tapering distally, slightly shorter than antepenultimate article, tip widened, with 13 spiniform setae; exopod slender, reaching to about 0.8 of antepenultimate article length.

Major cheliped (Fig. 6A–C) with coxa unarmed; basis and ischium short; merus robust, dorsal margin convex, about twice as long as broadest part, dorsodistal margin blunt; carpus short, cup-shaped; palm swollen, ovoid, about 1.9 times as long as broad, dorsodistal margin with small projection; fingers about 0.6 times as long as palm, pollex subequal to dactylus; plunger slightly reduced. Minor cheliped (Fig. 6D–F) with coxa unarmed; basis and ischium short; merus relatively robust, about 2.5 times as long as broadest part; carpus vase-shaped; palm ovoid, nearly 0.7 times as long as merus, about 0.7 times as long as broad; fingers subequal, about 0.8 times as long as palm, broadened, with minute accessory projection at tip; dactylus spoon-shaped.

Second pereiopod (Fig. 7A, B) with basis short; ischium relatively slender, approximately three times as long as broad; merus slender, five times as long as wide, about 1.5 times as long as ischium; carpus slender, about 1.4 times as long as merus, with four subdivisions, proximal

article longest, slightly longer than sum of other articles, second article slightly longer than third one, distal article about 2.7 times as long as third article; palm slightly longer than fingers; fingers very slender.

Three last pereiopods (Fig. 7C–E) shorter and more robust compared to general shape of walking legs in *Synalpheus*. Third pereiopod (Fig. 7C) robust, coxa armed with small spiniform setae on dorsodistal margin; ischium vase-shaped, slightly longer than widest part; merus robust, about 2.2 times as long as wide, about 2.6 times as long as ischium; carpus slightly widening distally, 1.5 times as long as ischium, with single spiniform seta on distoventral margin; propodus relatively robust, about 0.7 times as long as merus, with five spiniform setae on ventral margin in addition to distal pair; dactylus biunguiculate, superior unguis remarkably longer (around 3 times) than inferior one. Fourth pereiopod (Fig. 7D) generally similar to third pereiopod, slightly less robust; coxa lacking spiniform setae on dorsodistal margin. Fifth pereiopod (Fig. 7E) generally similar to third pereiopod, less robust; carpus about 0.7 times as long as merus, without spiniform setae on distoventral margin; propodus subequal to merus, bearing three spiniform setae on ventral

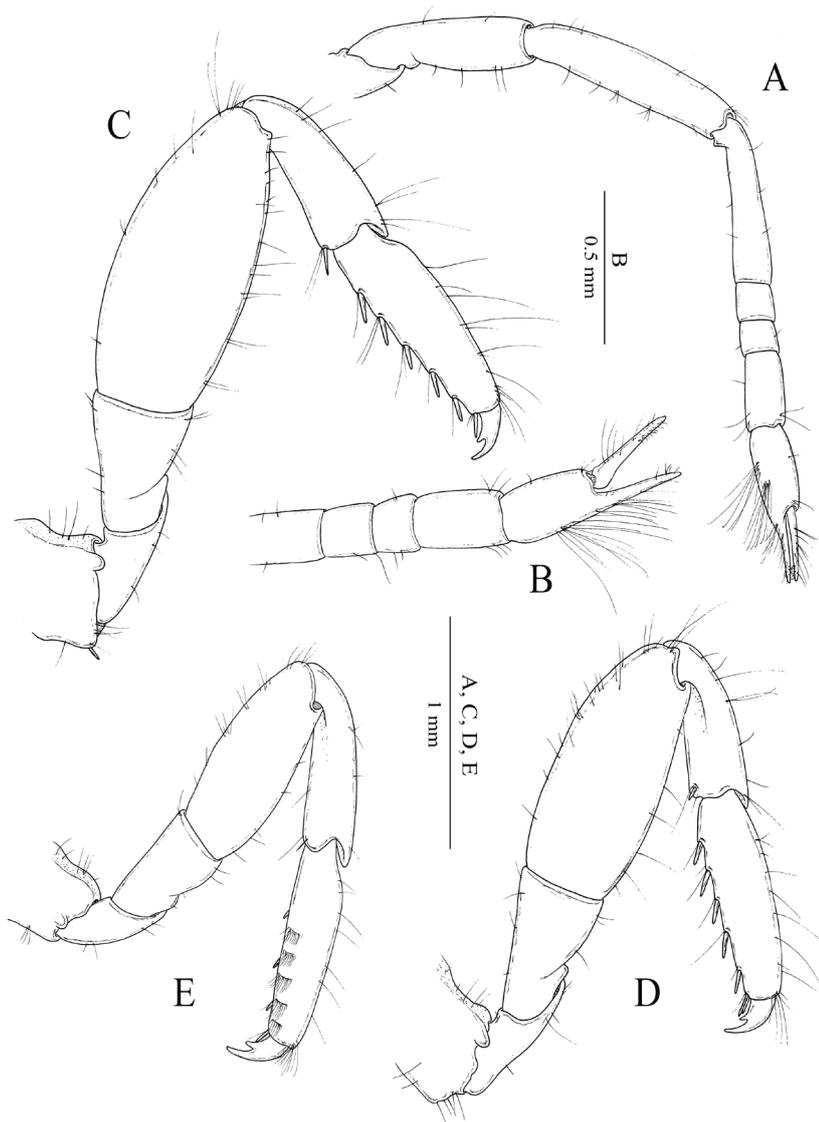


Figure 7. *Synalpheus gustavi* sp. nov., holotype (FLMNH-UF-71394). **A** second pereiopod, **B** same, distal segments, **C** third pereiopod, lateral view, **D** fourth pereiopod, lateral view, **E** fifth pereiopod, lateral view.

margin and one pair distally, furnished with five transverse rows of microserrulate setae on distal half.

Uropods (Fig. 5E) with mesial tooth of protopod blunt, lateral tooth subrounded with subdistal blunt projection on mesial side; exopod broad, with small distolateral tooth next to slender spiniform seta; diaeresis lacking except for lateral margin terminating to small tooth; endopod slightly longer than exopod.

Type locality. North of Madagascar: Nosy Vorona.

Ecology. All the specimens were collected from cryptic sponges situated among dead coral and coral rubble.

Remarks. The new species, *S. gustavi* sp. nov., shares a distinctive spoon-shaped feature of the minor cheliped, along with the presence of an orbitorostral process, with several species found in the Indo-Pacific region. These species include *S. anceps* AH Banner, 1956; *S. dorae* Bruce 1988; *S. harpagatrus* Banner and Banner, 1975; *S. laticeps* Coutière, 1905; *S. neptunus neptunus* (Dana, 1852); *S. neptunus germanus* Banner and Banner, 1975; *S. paradoxus* Banner and Banner, 1981; *S. paralaticeps*

Banner and Banner, 1982; and *S. theano* De Man, 1910. Among these morphologically similar species, we included *S. neptunus neptunus*, *S. anceps*, and *S. theano* in the phylogenetic tree to explore their relationship with the new species. However, the results of the phylogenetic analysis reveal that the spoon-shaped minor chela has evolved independently in *S. gustavi* sp. nov. compared to other Indo-Pacific species exhibiting similar characteristics in the minor chela.

The new species can be distinguished from the aforementioned species based on four distinct characteristics, allowing for a quick and accurate differentiation. These traits are as follows: 1) very shallow frontal margin of the carapace, both the rostrum and orbital teeth, 2) unique shape of the ultimate segment of the third maxilliped, 3) presence of a four-articled carpus in the second pereiopod, and 4) exceptionally narrow and slender fingers in the second pereiopod.

Synalpheus anceps, which was reported from Saipan in the Northern Mariana Islands (AH Banner 1956), is the only species, along with the new species, that possesses a very shallow frontal margin. However, there are notable differences between the two species. In *S. anceps*,

the front (including the rostrum and orbital teeth) is less pronounced, the blade of the scaphocerite is complete, the ultimate segment of the third maxilliped is normal *Synalpheus* like, the second pereopod carpus has five articles with relatively normal-shaped fingers, and the third pereopod is generally less robust compared to that of the new species. Additionally, in *S. anceps*, the superior unguis of the last three pereopods is slightly longer than the inferior unguis. On the other hand, the new species can be distinguished from *S. anceps* by having a squame reduced to a small projection, a distally broad ultimate segment of the third maxilliped, a four-articled carpus in the second pereopod with very slender fingers, and the three last pereopods with superior unguis of the dactylus significantly longer than the inferior ones.

Synalpheus doriae shares one of the four distinctive characteristics of *S. gustavi* **sp. nov.**, i.e. the second pereopod with four-articled carpus. However, there are several other notable differences between the new species and *S. doriae*: the less developed superior tooth of the basicerite in the new species compared to that of *S. doriae*, the more developed scaphocerite blade in *S. doriae*, the major chela palm terminating to a small projection dorso-distally in *S. gustavi* **sp. nov.** while bearing a prominent tooth in *S. doriae*, more robust three last pereopods with dorsal unguis about three times as long as ventral unguis in *S. gustavi* **sp. nov.** compared to slightly longer superior unguis in *S. doriae*, very strong dorsal spiniform setae of telson, and very narrow posterior margin of telson in *S. doriae* with mesial spiniform setae being juxtaposed.

Three species, namely *S. harpagatrus*, *S. laticeps*, and *S. paralaticeps*, are distinguished by the specific shape of the fingers on the minor cheliped, which possess 3 or 4 teeth at the tip. In contrast, the new species has a single tooth and a tiny accessory tooth on the fingers. Additionally, the following differences, in addition to the four main distinctions, can be observed between these three species and the new species: the longer stylocerite (overreaching the first antennular article) and more developed blade of the scaphocerite in the three of them compared to *S. gustavi* **sp. nov.**; typical ultimate segment of the third maxilliped in *S. laticeps* and *S. paralaticeps* compared to broad distally and armed with 13 slender spiniform setae in *S. gustavi* **sp. nov.**, and narrow distally armed with 10–12 short heavy spiniform setae in *S. harpagatrus*; stouter general shape of the minor cheliped in *S. harpagatrus*; armed merus of the third pereopod in *S. harpagatrus* and *S. paralaticeps* but unarmed in *S. laticeps* and *S. gustavi* **sp. nov.**; proportion of the superior unguis of the third pereopod dactylus to the inferior one being approximately 3 in *S. gustavi* **sp. nov.**, 2 in *S. paralaticeps* and 1 in *S. harpagatrus* and *S. latirostris*; fully developed diaeresis in *S. harpagatrus* and *S. paralaticeps* compared to the reduced one to a lateral tooth in *S. gustavi* **sp. nov.**; straight posterior margin of the telson in *S. gustavi* **sp. nov.** compared to the convex one in the other three species.

The Red Sea-inhabiting species, *S. paradoxus*, can be further distinguished from *S. gustavi* **sp. nov.** by the following characteristics, in addition to the four previously mentioned differences: its very strong superior tooth of

the basicerite; half-developed blade of the scaphocerite; major cheliped merus armed with a strong tooth dorso-distally, palm with strong tubercle on the dorso-distal margin, and the dactylus longer than the pollex; minor cheliped dactylus with two definite rows of setae situated dorsally and mesially; third pereopod dactylus with the superior unguis about twice as long as the inferior unguis; complete diaeresis; and triangular distolateral angles of the telson.

The three remaining species *S. neptunus neptunus*, *S. neptunus germanus* and *S. theano*, are separable from the new species by various characters, in addition to the four mentioned differences (De Man 1911; Banner and Banner 1972, 1975). Regarding the frontal appendages, *S. theano*, *S. neptunus neptunus*, and *S. gustavi* **sp. nov.** have a shorter stylocerite falling short of distal margin of the first antennular article while it overreaches the first antennular article in *S. neptunus germanus*; *S. theano* and *S. gustavi* **sp. nov.** possess a longer superior tooth of the basicerite reaching to about midlength of the second antennular article or end of it, while in the other two species it fails to reach the midlength of the second antennular article; the scaphocerite blade is absent in *S. neptunus germanus*, reduced to a small bump in *S. gustavi* **sp. nov.**, partially reduced in *S. neptunus neptunus*, and with no reduction in *S. theano*. Regarding the thoracic appendages, *S. neptunus neptunus* and *S. neptunus germanus* possess a typical shape of the ultimate segment of the third maxilliped, *S. theano* lacks the crown of spiniform setae replaced by setae, and that of *S. gustavi* **sp. nov.** is broadened distally with 13 spiniform setae; the major cheliped palm terminates to a tiny projection in *S. gustavi* **sp. nov.** while the other three to prominent tubercles; the minor cheliped dactylus is furnished with two rows of setae, one dorso-mesially and one mesially in *S. neptunus germanus*, while there is only one mesially located row of setae in *S. theano* and *S. neptunus neptunus*, and *S. gustavi* **sp. nov.** has no row of setae; the superior unguis of the third pereopod is remarkably longer in *S. gustavi* **sp. nov.** compared to the inferior unguis, whereas it is only slightly longer in the other three species. Regarding the pleonal appendages, three differences are notable. First, the diaeresis is complete in *S. theano* and *S. neptunus neptunus*, while it is limited to its lateral tooth in *S. neptunus germanus* and *S. gustavi* **sp. nov.** Second, the position of dorsal pairs of spiniform setae on telson are more mesially (especially the posterior pair) in *S. gustavi* **sp. nov.** (for detailed comparison see De Man 1911: fig. 61a for *S. theano*, Banner and Banner 1972: fig. 3M for *S. neptunus neptunus*, Banner and Banner 1975: fig. 12i for *S. neptunus germanus*, and Fig. 5E for *S. gustavi* **sp. nov.**). Third, the posterior margin of the telson is straight in *S. gustavi* **sp. nov.**, almost straight in *S. neptunus germanus*, and concave in *S. theano* and *S. neptunus neptunus*.

Phylogenetically speaking, *S. gustavi* **sp. nov.** was initially included in the first and only worldwide phylogenetic study of the genus *Synalpheus* (Hultgren et al., 2014) as *S. aff. brevisfrons*. The new species is broadly grouped with the *S. gambarelloides* species group, consistent with the findings of the present study.

4. Discussion

Since the initial documentation of eusociality in *Synalpheus* shrimps, prerequisites for the evolution of eusociality, as well as numerous distinctive characteristics unique to eusocial species have been reported and extensively discussed (for instance, Duffy 2007; Tóth and Bauer 2007; Tóth and Duffy 2008). According to the ‘Fortress Defence Hypothesis’ proposed by Crespi (1994), eusociality can arise under three sufficient conditions. As discussed by Duffy (2007), the host sponges inhabited by *Synalpheus* shrimps fulfil the first condition by providing both food (long-lived resources) and shelter, making them valuable resources for defence and facilitating specialization in the protection of kin. The shrimps’ lifelong association with sponges has led to the development of various adaptations for feeding. Among the diverse shapes observed in the minor cheliped, a specialized feeding appendage, only two forms are found in eusocial species. The first form is the characteristic shape of the minor cheliped in the *S. gambarelloides* group, wherein the dactylus is adorned with densely located rows of setae (commonly known as *gambarelloides* setae). This form predominates in species of the *S. gambarelloides* group. The second form is characterized by spoon-shaped fingers in the minor cheliped and is observed in species of the *S. neptunus* group, *S. paraneptunus* group, and several other species in the Indo-Pacific region. When compared to other forms of minor chelipeds, these two forms can be regarded as the most advanced evolutionary adaptations among sponge-dwelling *Synalpheus*. It should be noted that while the first form (*S. gambarelloides* form) is exclusive to sponge-dwellers, the second form is not limited to them. For instance, certain types of pair-living *S. neptunus* are observed to inhabit coral burrows and crevices, displaying this particular form of minor cheliped. Although the *gambarelloides* type of minor cheliped is the predominant form in Atlantic *Synalpheus* species, it is relatively rare in the Indian and Pacific oceans (see the ‘remarks’ section under *S. sponjy* **sp. nov.**).

One of the most remarkable examples of division of labor was observed by Duffy (1999) in larger females of *S. filidigitus* Armstrong, 1949. While smaller females maintain a morphology similar to other colony members, the larger females were reported to lack the massive major cheliped (their defensive tool) and instead rely on two minor chelipeds (their feeding tools), representing a striking transition towards division of labor. Interestingly, the first observation of females with two minor chelipeds in the Indo-Pacific species of *Synalpheus* was documented by Banner and Banner (1983) in the colony of *S. crosnieri*. In this colony, a total of 147 specimens were reported, with only three females (two of them ovigerous) possessing exclusively minor chelipeds. Two of the females had two minor chelipeds present, while the third female had one of the first pereopods missing. Another observation made by Banner and Banner (1983) regarding *S. crosnieri* was the relatively large size of the eggs, measuring approximately 0.6 mm in diameter. Considering all the

aforementioned characteristics of *S. crosnieri*, this species is undeniably a eusocial species.

Synalpheus sponjy **sp. nov.** not only represents the species that is morphologically closest to *S. crosnieri* but also exhibits a similar life style. While no females were found in the collected colonies of the new species, the assemblages of the collected specimens within a sponge varied between 1 and 19 males. Like other eusocial species of *Synalpheus*, this species inhabits cryptic sponges situated among dead corals, making it challenging to sample the entire sponge (and the entire colony) during a typical survey of coral rubble. However, considering the number of collected specimens (24 males), and the fact that 19 of them were collected from a single sponge, the most likely conclusion is that *S. sponjy* **sp. nov.**, like its sister taxon, is a eusocial species.

In contrast to the rarity of the *gambarelloides* small chela type in Indo-Pacific species, the prevalence of the spoon-shaped type of chela is notable in this region, with several species showing indications of evolving a eusocial system. One such species is *S. neptunus germanus*, described by Banner and Banner in 1975 based on 44 specimens collected from three different sites. Although all specimens were reported as juveniles, there are indications that the species is eusocial based on personal observations of the authors. Furthermore, eusociality has been observed in at least one additional species within the *S. neptunus* group. Didderen et al. (2006) reported four colonies of *S. neptunus* from Indonesia, varying in size, with the largest colony consisting of up to 388 individuals within a single sponge. The morphology and ecology of the *S. neptunus* complex will be further discussed in forthcoming studies (Ashrafi et al.). Another eusocial species with the characteristic spoon-shaped fingers of the minor cheliped is *Synalpheus paradoxus*, described from the Red Sea (Banner and Banner 1981). The species name “paradoxus” emphasizes the highly skewed sex ratio observed in this species. Colonies of *S. paradoxus* were found in three different sponges, with colony sizes ranging from 113 to 132 individuals, each containing only two ovigerous females.

Lastly, the newly discovered species, *S. gustavi* **sp. nov.**, represents another eusocial species characterized by the spoon-shaped fingers of the small chela. Alongside the number and size of eggs, *S. gustavi* **sp. nov.** exhibits one of the most significant hallmarks of eusociality—a skewed sex ratio. Colony sizes ranged from 1 to 19 individuals, although it is possible that some records of solitary individuals originated from individuals separated from the rest of their colonies within the rubble. Among colonies with females, the sex ratio varied from 1/3 (UF 14231) to 1/19 (UF 14259, including the allotype ovigerous queen). Interestingly, colonies of *S. gustavi* **sp. nov.** showed a tendency for high rates of parasitism (primarily in the pleonal area) by bopyrid isopod parasites. The rates of individual lot parasitism ranged from 0% to 50% (mean = 14.4%), comparable to the parasitism rates reported in some eusocial *Synalpheus* colonies by Duffy (1992) (30% in *S. brooksi*) and (McGrew and Hultgren, 2011) (25% in *S. elizabethae*).

In summary, the two new species, *S. sponjy* **sp. nov.** and *S. gustavi* **sp. nov.**, exhibit assemblages indicative of a eusocial colony structure. These include the presence of large numbers of male (non-ovigerous) individuals residing together in a single sponge, with one or zero females present. Additionally, the two new species share ecological and morphological characteristics with other described eusocial *Synalpheus* species. Firstly, both species inhabit sponges, a symbiotic lifestyle believed to be a prerequisite for the evolution of eusociality. Moreover, the presence of multiple size classes, including juveniles, within the same sponge suggests overlapping generations, further supporting their eusocial nature. Secondly, similar to eusocial species in the West Atlantic, the two new species are relatively small-bodied (mean CL of *S. gustavi* **sp. nov.** = 2.62, mean CL of *S. sponjy* **sp. nov.** = 2.93), comparable to the body size of other described eusocial species (mean = 3.2 mm CL), but smaller than the average body size of communal species (mean CL = 5.32) and pair-living species (mean CL = 5.57) (Hultgren et al. 2021).

5. Conclusion

Eusociality has been extensively documented and studied in several Atlantic species of *Synalpheus*. However, numerous Indo-West Pacific species of *Synalpheus* with a high likelihood of possessing a eusocial living style have remained unnoticed, as they were described and/or reported prior to 1996 when eusociality was first reported in *Synalpheus* by Duffy in 1996.

The recent descriptions of two new species and the presence of at least four other eusocial species in the Indo-West Pacific region emphasize the likelihood of multiple independent evolutions of eusociality within the genus *Synalpheus*. These findings highlight the importance of conducting taxonomic and ecological studies on species within this region.

It is important to acknowledge the significant contributions made by Coutière, De Man, and Banner and Banner in their work before 1986, which greatly enhanced our understanding of *Synalpheus* species in the Indo-West Pacific. However, it is evident that earlier studies did not emphasize eusocial behavior and overall living styles, primarily due to the lack of recognition of eusociality before Duffy's publication in 1996. The limited taxonomic and ecological studies on Indo-West Pacific species of the genus indicate a noticeable gap in our understanding. The species richness of Indo-West Pacific *Synalpheus*, particularly in terms of eusocial species, is currently underestimated. Therefore, it is crucial to conduct further research and observations to deepen our understanding of the taxonomy, morphology, and ecology of this fascinating genus. Continued research efforts will undoubtedly illuminate the complex behaviors, social structures, and ecological interactions of *Synalpheus* species not only in the Indo-West Pacific region but also in other regions,

especially poorly-studied ones such as the East Pacific. Ultimately, this will lead to a more comprehensive understanding of this remarkable group of organisms.

6. Author's contributions

HA: Conceptualization, Methodology, Original draft.

KH: Conceptualization, Methodology, Review and Editing.

7. Funding

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8. Competing interests

The author has declared that no competing interests exist.

9. Availability of Data

All the sequences used in the study are submitted to the GenBank with the numbers provided in the Table 1.

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Supplementary Material 1

Fig. S1

Authors: Orlandin E, Piovesan M, Herbin D, Carneiro E (2023)

Data type: .tif

Explanation note: Bayesian gene tree based on COI sequences. Numbers above clades indicate Bayesian posterior probabilities. Taxon labels are given as *Synalpheus* species, locality, and genetics identifier (given in Table S1). New species described in this study are figured in red.

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