Journal of Crustacean Biology

Research Note A Newly Described Color Morph of Calappa flammea (Herbst 1794) (Decapoda, Calappidae) with Taxonomic Implications for the Genus Calappa

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Note
Section/Category:	Phylogeny and Taxonomy
Keywords:	box crabs; shame-faced crabs; Cytochrome Oxidase 1; museum collection
Corresponding Author:	Jason Macrander Florida Southern College Lakeland, Florida UNITED STATES
First Author:	Jason Macrander
Order of Authors:	Jason Macrander
	Lennart Vossgaetter
	Paul Larson
Manuscript Region of Origin:	UNITED STATES
Abstract:	Crustaceans in the family Calappidae (Malacostraca, Decapoda) are commonly known as ‘box crabs' or ‘shame-faced crabs.’ Many species of Calappidae look alike and are difficult to distinguish at various developmental stages based on morphology alone. Some crustaceans recently collected as a part of the South East Atlantic Monitoring and Assessment Program (SEAMAP) groundfish surveys could not be determined to species due to their unusual color pattern and size; however, they were identified as likely members of the family Calappidae. The present study aims to identify this unique color morph taxonomically and morphologically. The use of molecular data in combination with morphometrics suggests that the crabs in question were Calappa flammea . Our results show that these specimens represent an undescribed unique color morph that deviates from their previous description and should be considered for future marine benthic surveys. Several ecological factors can cause such color variations in crabs, but to understand the drivers the described phenotypic variance in C. flammea needs further investigation.

Cover Letter

Dr. Jason Macrander
Assistant Professor of Marine Biology
Florida Southern College
111 Lake Hollingsworth Drive, Lakeland, FL 33801
863.680.4351
jmacrander@flsouthern.edu

March 8, 2021

Dear Dr. Fernando L. Mantelatto,

I am delighted to submit for consideration our manuscript titled "A Newly Described Color Morph of *Calappa flammea* (Herbst 1794) (Decapoda, Calappidae) with Taxonomic Implications for the Genus *Calappa*." Our study uses a combination of morphological and molecular (CO1) data to determine the taxonomic designation of a previously unidentified and ambiguous morphotype of box crab found off the coast of Florida. The specimens were originally collected by the Florida Fish and Wildlife South East Atlantic Monitoring and Assessment Program (SEAMAP) groundfish surveys, with some specimens used in our study dating back to 1973. Our study revealed that a unique morphotype of this box crab that had been collected by SEAMAP groundfish surveys was mis-identified for decades, wrongly attributed to various other local Calappid species. Even though the coloration and tuberculation patterns differ, a combination of molecular and morphological analyses provides strong support for these unique morphotypes to be designated as *Calappa flammea*. It remains to be determined the source of the contrasting morphotypes in this species, however, the importance of fine scale anatomical analyses, museum collections, and molecular analyses in resolving species identification is evident in our study. For the reasons outlined above, we feel it would be of interest to readers of Journal of Crustacean Biology as a research note. We look forward to hearing from you, please let me know if you have any questions or concerns.

Sincerely,

Jason Macrander

Jason Macrander, PhD

RUNNING HEAD: COLOR MORPH OF *CALAPPA FLAMMEA*

Research Note

A Newly Described Color Morph of *Calappa flammea* (Herbst 1794) (Decapoda, Calappidae) with Taxonomic Implications for the Genus *Calappa*

Lennart Vossgaetter[(1)], Paul Larson[(2)], Jason Macrander[(1)]

[(1)] *Biology Department, Florida Southern College, 111 Lake Hollingsworth Drive, Lakeland, FL, 33815, USA*

[(2)] *FWC Fish and Wildlife Research Institute, 100 8th Ave SE, St., St. Petersburg FL, 33701, USA*

Correspondence*: Jason Macrander, jmacrander@flsouthern.edu*

Abstract Crustaceans in the family Calappidae (Malacostraca, Decapoda) are commonly known as ‘box crabs' or ‘shame-faced crabs.’ Many species of Calappidae look alike and are difficult to distinguish at various developmental stages based on morphology alone. Some crustaceans recently collected as a part of the South East Atlantic Monitoring and Assessment Program (SEAMAP) groundfish surveys could not be determined to species due to their unusual color pattern and size; however, they were identified as likely members of the family Calappidae. The present study aims to identify this unique color morph taxonomically and morphologically. The use of molecular data in combination with morphometrics suggests that the crabs in question were *Calappa flammea*. Our results show that these specimens represent an undescribed unique color morph that deviates from their previous description and should be considered for future marine benthic surveys. Several ecological factors can cause such color variations in crabs, but to understand the drivers the described phenotypic variance in *C. flammea* needs further investigation.

Key words box crabs, shame-faced crabs, Cytochrome Oxidase 1, museum collection

Crustaceans in the family Calappidae (Malacostraca, Decapoda) are commonly known as ‘box crabs' or ‘shame-faced crabs’. The family includes eight genera and >40 species distributed in tropical and subtropical waters. The defining characteristics of these crabs are their enlarged chelae and a unique feeding strategy that enables these crabs to feed on gastropods (Bellwood 1998, Shoup 1986, Ng et al. 2002). Despite recent efforts using morphological (Bellwood 1996), fossil (Schweitzer and Feldmann 2000), and molecular data (Lai et al. 2006, Ewers-Saucedo et al. 2016), the taxonomy remains somewhat ambiguous and unresolved as many Calappidae species are similar in appearance and difficult to distinguish at various developmental stages based on morphology alone (Schweitzer and Feldmann 2000, Lai et al. 2006, Kumar et al. 2013).

During recent South East Atlantic Monitoring and Assessment Program (SEAMAP) groundfish surveys, specimens obtained were determined to belong to the family Calappidae; however, they could not be identified to species level due to their unusual color pattern. There are currently thirteen species reported off the coast of Florida within the family Callapidae (Abele and Kim 1986, Felder et al. 2009) and although these specimens somewhat resembled *C. flammea* the unique color morph was consistently smaller in size. In contrast to the typical purplish-brown interlacing bands and longitudinal stripes (Williams 1984) of *C. flammea* the unidentifiable crabs instead exhibited a light beige base coloration with blotches of light orange-brown distributed throughout (Fig. 1). In addition, these lacked the prominent dark spots which are present on the chelae of *C. flammea* (Fig. 1. B, E), exhibited heavier tuberculation on the chelae and carapace (Fig. 1 A, D), and had a more acutely pointed toothed spine on the proximolateral margin of the propodus (Fig. 1. C, F). Given the differences in color pattern, size range, and textures in these specimens,

those newly collected specimens not matching the traditional *C. flammea* characteristics and were considered to be potentially a new species of *Calappa* and set aside with the temporary identification of *Calappa* 'sp. E,' which is the name that will henceforth be used to refer to these individuals. A subsequent re-evaluation of all of the cataloged *Calappa* material (n = 426 lots) in the Florida Biodiversity Collection at Florida Fish and Wildlife Research Institute (FWRI, St. Petersburg, FL) uncovered more *C.* 'sp. E'- like individuals with questionable identifications. Most of these were originally identified only to the genus *Calappa*, with the remainder having been assigned to various other local Calappid species (i.e., *C. sulcata, C. ocellata, C. flammea*, and *Calappula tortugae*).

In this study, we aim to determine the taxonomic designation of the unique color morph recovered in the Gulf of Mexico using a combined morphological and molecular data sets. Their coloration and shape were examined alongside the most morphologically similar species, *C. flammea*, and other members of the Calappidae family. Their trawling locations were also evaluated to determine if their geographic distribution or depth could explain the unique color morph.

Specimens sampled for genetic analysis were collected between 1981 and 2019 (GenBank Accessions: MW412185-MW412239). Specimens used for morphological comparisons were collected between Oct 09 1973 and Oct 27 2019 (Supplemental File 1). All specimens used for morphology were stored in 70% EtOH with the older specimens (prior to ~2012) having been formalin-fixed. Not every specimen used in the morphological analysis was used in our genetic analysis due to the preservation and storage conditions. Tissues collected were either processed and sequenced for FWRI by the Canadian Centre for DNA Barcoding (Guelph, Ontario, Canada) according to internal protocols or at Florida

Southern College using the DNEasy kit (QIAGEN, Hilden, Germany) and puReTaq Ready-To-Go PCR Beads (Cytiva, Marlborough, MA) following the PCR Protocol in Ewers-Saucedo (2016). Newly acquired sequences, along with publicly available data from Genbank and the Barcode of Life Database (www.boldsystems.org), were aligned in MAFFT using the L-INS-i strategy (Katoh et al. 2005). A CO1 phylogenetic tree reconstruction was conducted in MEGA version X using the Maximum Likelihood method with 1000 bootstrap replicates under the GTR+G+I model, as determined from the likelihood values of the best fit model (Kumar et al. 2018, Stecher et al. 2020, Nei and Kumar, 2000). Positions with less than 50% coverage were not used for the final phylogenetic tree reconstruction. A haplotype network of just the *Calappa* 'sp. E' clade was reconstructed in a minimum spanning network (Bandelt et al. 1999) in the program PopART (http://popart.otago.ac.nz) to determine if there was a genetic structure between the west and east coast of Florida.

Measurements of carapace width and length were taken of unidentified specimens (*C.* 'sp. E') and *C. flammea*. The maximum length was measured from the rostrum to the posterior end of the carapace. The width was measured from the third groove (from posterior towards anterior) of the posterolateral teeth. The sex and color patterns were noted based on species descriptions (Holthius, 1958). Unquantifiable characteristics that appeared unique were noted and confirmed by FWRI collections staff. Photographs were taken with a DSLR camera to visualize the differences in color patterns. The carapace sizes were compared with the Mann-Whitney U test to adjust for a non-parametric distribution. The allometric relationship between carapace width and length was tested with the analysis of covariance (ANCOVA) to compare the slopes of the regression lines and the overall mean

value based on differences in intercepts (Packard and Boardmann 1988). The trawling depths at which the individuals were caught were compared with a Student's t-test. The trawling locations were compared for both groups in QGIS to assess if there was any geographical isolation. The statistical tests were conducted in IBM SPSS Statistics.

Our newly collected sequence data in combination with previously published sequences consisted of 89 nucleotide sequences across 627 sites. The highest log likelihood (-7352.12) tree is shown (Fig. 2 A). Except for the clade consisting of *C.* 'sp. E' and *C. flammea*, the resulting phylogenetic tree produced morphologically distinct lineages with high bootstrap support value belonging to morphologically distinct species-level clades. The clade composed of *C.* 'sp. E' and *C. flammea* were supported by a bootstrap value of 100% and individuals of either color morph do not segregate based on their nucleotide sequences with strong support for *C. ocellata* to be sister to the *C. flammea*/Sp. E clade (Fig. 2 A). Nucleotide diversity within the clade 'sp. E' had a total of 31 segregating sites, 12 of which were parsimony informative. Overall there was low nucleotide diversity (n = 0.00467) and a negative (-2.29) Tajima's D statistic, which is indicative of the abundant rare alleles. The minimum spanning haplotype network for the 'sp.E' clade did not support any strong genetic structure between the west and east coast of Florida (Fig. 2 B). Both, *C. flammea* and *C.* 'sp. E' were caught on the west and east coast of Florida in the Atlantic and the Gulf of Mexico. The trawling locations did not show any geographical isolation between the two groups or any aggregations with only one group present except for one individual of *C. flammea* caught in deeper water in the Gulf of Mexico (Fig. 2 C).

Morphological data were collected for 56 *C.* 'sp. E' and 31 *C. flammea.* Of the 56 *C.* 'sp. E', 32 were male and 22 were female, one remained un-scored due to a missing

abdomen. Sixteen were male and 15 were female of the 31 *C. flammea.* Even though we tried to only take data of the smaller *C. flammea,* they were significantly larger than the *C.* 'sp. E' (Z=-4.091, P=0.000). The largest measured carapace length for a *C.* 'sp. E' was 45.20mm, whereas the mean was 20.89mm. The allometric relationship of width and length between the two groups is described by the linear equations $Width_{flammea}$ = 1.399 * Length - 4.361 and $Width_{speciesE}$ = 1.285 * Length - 1.667. The relationship is statistically different (F = 41.30; df = 1, 83; p = 7.8 x 10^{-9}) (Supplemental File 1). The intercepts are not statistically significant (F = 1.13, p = 0.29).

When evident, the color pattern was used consistently to differentiate between the two groups (Fig. 1). When faded due to preservation, specimens were differentiated based on tuberculation and spination characteristics of each species. Most *C. flammea* specimens showed red flame-like lines leading posterior to anterior along its pale to light-orange carapace, whereas all (N=31) displayed the flame-like lines at least on their chelae (Fig. 1, C). None (N = 0) of the *C.* 'sp. E' were showing any evidence of these flame-like lines. Their carapace was uniformly colored in a beige tone with orange to dark brown blotches that appeared somewhat pink to violet in some specimens. The brightness of the color was varying along the carapace with no apparent patterns (Fig. 1 D, E,F,). Some specimens (N = 26) of the *C.* 'sp. E' were pale, likely due to long preservation times, and no color patterns could be determined. Among these two groups, we observed a consistent difference in the prominence of the tubercles on the carapace and chelae, where *C.* 'sp. E' has more prominent tubercles that extend outward at a greater slope (Fig. 1 D,E,F). *C.* 'sp. E' showed a more acutely pointed toothed spine on the proximolateral margin of the propodus (Fig. 1 D,E,F).

Even though *C. flammea* and *C.* 'sp. E' from the collection differ in their color patterns and prominence of the tubercles on the carapace and chelae, the molecular data from the partial CO1 fragment showed that these two groups fall in the same clade with strong bootstrap support, likely all being *C. flammea* (Fig. 2 A). The color pattern deviates from the description of *C. flammea* in that their carapace was uniformly colored in a light beige with blotches of light orange-brown distributed throughout. Although lack of color could be attributed to long preservation time, this was not the case for all specimens designated as *C.* 'sp. E'. Their tuberculation was more prominent on carapace and chelae and the toothed spine on the proximolateral margin of the propodus was more acutely pointed (Fig. 1 C,F). Furthermore, based on trawling locations and depths, there was no spatial separation apparent between the two morphs. The specimens with the unique morphology likely belong to the species *C. flammea*, but exhibit a morphotype that has thus far evaded description. Despite the unique coloration used to identify clade 'sp.E', there is no support that this unique color morph should be separate from the unambiguously identified *C. flammea*.

Many specimens within the family Calappidae have been classified as juvenile morphotypes when they were simply different species (Ng et al. 1999). These conflicting designations are further perpetuated with age-specific color patterns and morphotypes, along with differing preservation and storage methods, which may be the source of some previous Callapidae taxonomic descriptions (Galil and Clark 1994, Galil 1997, Ng et al. 1999, Ng et al. 2002). In general, color and pattern have a variable degree of taxonomic importance to species circumscription across taxa, and color changes over time in preserved specimens can be alternately a problematic or useful occurrence. Simmons (2014) gives

examples in which color changes caused by fluid preservatives have revealed cryptic species in fish on one hand, and led to the erroneous description of junior synonyms in birds on the other.

Based on our CO1 tree reconstruction, *C. ocellata* appears to be most closely related to *C. flammea*. However, our limited taxonomic sampling did not include *C. cinerea* or *C. convexa*, two species that appear to be more closely related to *C. flammea* than *C. ocellata* (Ewers-Saucedo et al. 2016). Holthius (1958) described *C. ocellata* as only having minor morphological differences from *C. flammea*, such as the carapace width to length ratio and more slender pointed tips of the anterolateral teeth. Knowledge of these subtle morphological differences has not been recorded in the taxa missing from our analysis, emphasizing the importance of combining morphological and molecular data in future Callapidae studies.

There were no specimens classified as a *C.* 'sp. E' in the FWRI collection (N=56) that had a greater carapace length than 45.20 mm. On average, they were 20.89mm ± 0.847mm (mean ± St. Dev.) large, whereas *C. flammea* is known to grow at least 106mm in carapace length (Holthius, 1958, FWRI museum collection). It remains uncertain if larger specimens of *C.* 'sp. E' exists or if *C. flammea* experiences ontogenetic color changes. However, this has never been reported in any Calappidae species to our knowledge. In other crabs, color variation has been shown to vary across their distribution (Yoshikawa et al. 2018), growth stage (Rahayu and Forest 1999), seasonal vulnerability to visual predators (Krause-Neehring et al. 2010), and specific habitat's substrate (Todd et al. 2005). Potentially, *C. flammea*'s polymorphism could relate to a similar ontogenetic shift in their habitat use, where juveniles occupy different environments causing an adaption of their

color pattern as a response to different ecological and environmental stressors. Further research needs to be conducted to identify possible reasons behind the color variation. The addition of population-level loci on these color morphs could clarify if the observed variation is due to population structure that evaded our analysis, a developmental trait, or potentially phenotypic plasticity. Ecological and ontogenetic data could assist with determining if these two color morphs are the byproducts of environmental factors or simply phenotypic changes during development.

Acknowledgements

The problem investigated here was first brought to our attention by Janessa Cobb and Kelly Hoolihan (FWRI). They, with Haley Holloway (FWRI), provided identifications and re-examinations of questionable and unidentified *Calappa* specimens from the Florida Biodiversity Collection. We thank Dr. Christopher Brandon, Dr. Nancy Morvillo, Dr. Eric Kjellmark, Dr. Christy Wolovich for assisting with statistical analysis and experimental design guidance. This study was supported by Florida Southern College.

References

Abele LG, Kim W. 1986. An illustrated guide to the marine decapod crustaceans of Florida: 1-760. Florida State University, Tallahassee.

Bandelt H, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**:37–48.

Bellwood O. 1996. A phylogenetic study of the Calappidae H. Milne Edwards 1837 (Crustacea: Brachyura) with a reappraisal of the status of the family. *Zoological Journal of the Linnean Society*, **118**:165-193.

Bellwood O. 1998. The phylogeny of box crab genera (Crustacea: Brachyura: Calappidae) with notes on their fossil record, biogeography and depth distribution. *Journal of Zoology*, **244**:459-471.

Felder DL, Alvarez F, Goy JW, Lemaitre R. 2009. Decapoda (Crustacea) of the Gulf of Mexico, with comments on the Amphionidacea, Pp. 1019-1104 in Felder, D. L. and Camps, D. K. (eds.), Gulf of Mexico-origins, waters, and biota. Biodiversity. Texas A&M University Press, College Station, Texas.

Galil BS, Clark PF. 1994. A revision of the genus Matuta Weber, 1795 (Crustacea: Brachyura: Calappidae). *Zoologische Verhandelingen, Leiden*, **294**:1–55.

Galil BS. 1997. Crustacea Decapoda: A revision of the Indo-Pacific species of the genus *Calappa* Weber, 1795 (Calappidae). *In:* A. crosner (ed.), Résultats des campagnes Musorstom, Volume 18. Mém. Mus. Natn. Hist. Nat. **176**:271-335.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research,* **33**:511-518.

Krause-nehring J, Starck JM, Palmer AR. 2010. Juvenile colour polymorphism in the red rock crab, *Cancer productus*: patterns, causes, and possible adaptive significance. *Zoology*, **113**:131-139.

Kumar BA, Kumar MS, Galil BS. 2013. Calappid and leucosiid crabs (Crustacea: Decapoda: Brachyura) from Kerala, India, with the description of a new species of *Mursia* Desmarest, 1823, from the Arabian Sea and redescription of *M. bicristimana* Alcock Anderson, 1894. *Zootaxa*, **3746**:529–551.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**:1547-1549.

Lai JCY, Chan WK, Ng PKL. 2006. Preliminary molecular and morphological study of the *Calappa lophos* species group (Decapoda:Brachyura:Calappidae). *Journal of Crustacean Biology*, **26**:193-205.

Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Ng PKL, Chen K-l, Chan T-y. 1999. Taxonomic notes on three Indo-West Pacific species of Calappa (Decapoda: Brachyura: Calappidae). *Raffles Bulletin of Zoology*, **47**:607–616.

Ng PKL, Lai JCY, Aungtonya C. 2002. The box and moon crabs of Thailand, with description of a new species of *Calappa* (Crustacea: Brachyura: Calappidae, Mutatidae). *Phuket Marine Biological Center Special Publication*, **23**:341-360.

Packard GC, Boardmann TJ. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. *Physiological Zoology*, **61**:1-9.

Rahayu DL, Forest J. 1999. Sur le statut de Calcinus gaimardii (H. Milne Edwards, 1848) (Decapoda, Anomura, Diogenidae) et description de deux espèces nouvelles apparentées. *Zoosyst*, **21**:461-472, figs. 1-4

Schweitzer CE, Feldmann RM. 2000. New species of calappid crabs from western North America and reconsideration of the calappidae sensu lato. *Journal of Paleontology*, **74**:230-246.

Shoup JB. 1968. Shell opening by crabs of the genus *Calappa*. *Science*, **160**:887-888.

Simmons JE. 2014. Fluid Preservation: A Comprehensive Reference. Rowman & Littlefield, Lanham, MD, pp 437.

Stecher G, Tamara K, Kumar S. 2020. Molecular evolutionary genetics analysis (MEGA) for macOS. *Molecular Biology and Evolution*, **37**:1237-1239.

Todd PA, Briers RA, Ladle RJ, Middleton F. 2005. Phenotype-environment matching in the shore crab (*Carcinus maenas*). *Marine Biology*, **148**:1357-1367.

Yoshikawa A, Nakano T, Satoh TP, Asakura A. 2018. A colour variation of *Clibanarius virescens* (Krauss, 1843) (Decapoda, Anomura) collected from Amami Oshima Island and Okinawa, Japan. *Crustaceana*, **91**:85-101.

Figure Captions

Figure 1. A. *Calappa flammea* (Herbst 1794), FSBC I 139334, Dorsal view, Scale = 45mm; **B.** FSBC I 139334 anterior view; **C.** FSBC I 139334 proximolateral spine (circled) on propodus of left chela. **D.** *Calappa* 'sp. E,' FSBC I 139327 dorsal view, scale = 35mm; **E.** FSBC I 139327 anterior view; **F.** FSBC I 139327 proximolateral spine (circled) on propodus of right chela.

Figure 2. A. Phylogenetic tree of CO1 gene by Maximum Likelihood (-7352.12). Bootstrap values of less than 50 are not shown. Genbank IDs with the * indicate the *C.* 'sp. E' morphotype. Underlined labels indicate sequences were derived from the Barcode of Life Database. **B.** Minimum spanning haplotype network of *C. flammea*/*C.* 'sp. E' clade spanning eastern and western distribution of sampling off the coast of Florida. **C.** Trawling locations where *C. flammea* and *C.* 'sp. E' were caught, noting the east/west separation and depth, lacking geographical isolation for both.

Figures

Figure 1

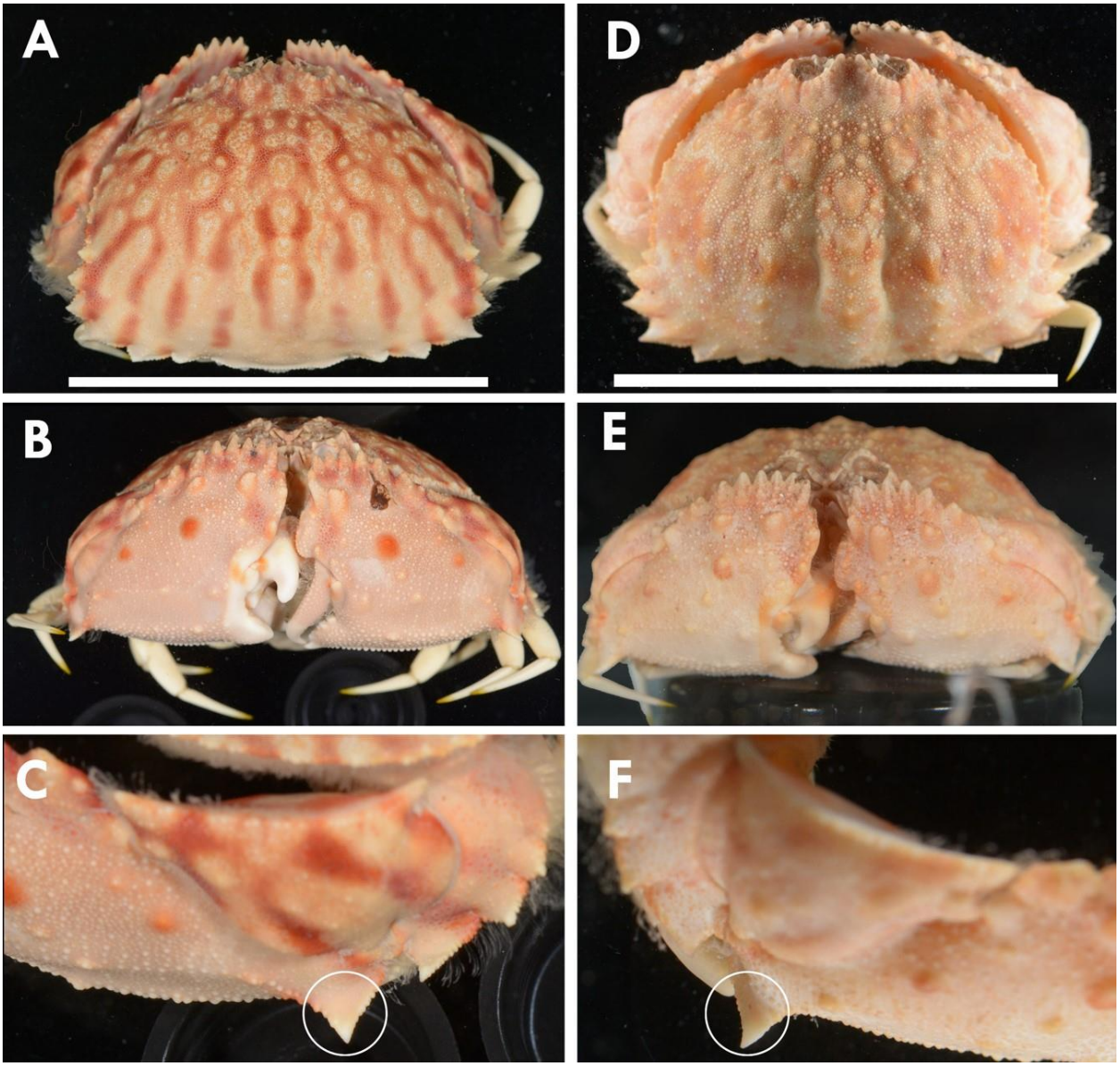
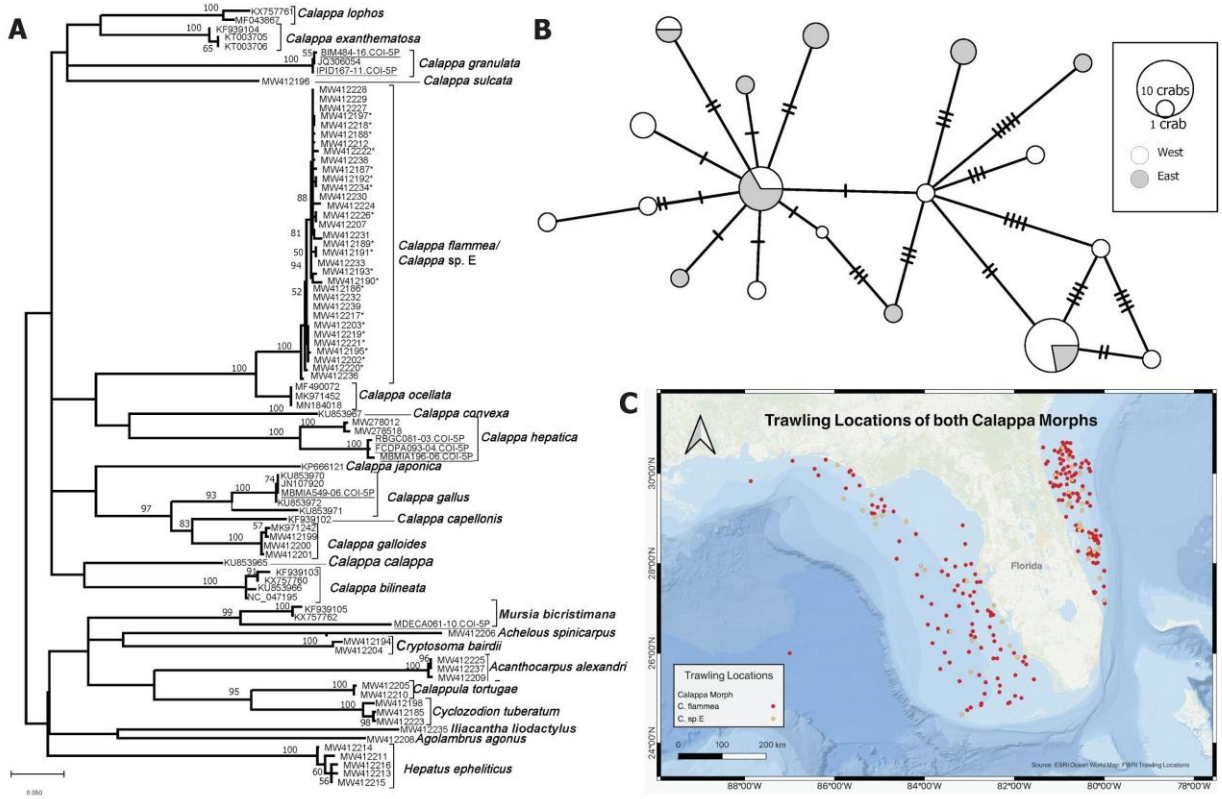


Figure 2


A
Calappa lophos
Calappa exanthematosa
Calappa granulata
Calappa sulcata
Calappa flammea/
Calappa sp. E
Calappa ocellata
Calappa convexa
Calappa hepatica
Calappa japonica
Calappa gallus
Calappa capellonis
Calappa galloides
Calappa calappa
Calappa bilineata
Mursia bicristimana
Achelous spinicarpus
Cryptosoma bairdii
Acanthocarpus alexandri
Calappula tortugae
Cyclozodion tuberatum
Iliacantha liodactylus
Agolambrus agonus
Hepatus epheliticus
B
10 crabs
1 crab
West
East
C
Trawling Locations of both Calappa Morphs
Florida
Trawling Locations
Calappa Morph
C. flammea
C. sp.E
0
100
200 km
30°0'0"N
28°0'0"N
26°0'0"N
24°0'0"N
88°0'0"W
86°0'0"W
84°0'0"W
82°0'0"W
80°0'0"W
78°0'0"W