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Author(s): Nathan Hollenbeck and David Scheel
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Body patterns of the frilled giant Pacific octopus, a new species of octopus from Prince William Sound, AK

Nathan Hollenbeck and David Scheel

Alaska Pacific University, 4101 University Dr., Anchorage AK 99508, U.S.A. nhollenbeck@alaskapacific.edu, dscheel@alaskapacific.edu

Abstract: We tested whether body patterns distinguished two haplotypes of large octopus in Prince William Sound Alaska. Live octopuses were photographed in captivity and assigned to a morphotype based on whether longitudinal mantle folds (a characteristic body pattern feature of the giant Pacific octopus (GPO), *Enteroctopus dofleini* (Wülker 1910)) were present (the GPO morphotype, N = 14) or absent (a novel morphotype, N = 6). Novel morphotype octopuses were distinguished without exception from GPO morphotype octopuses by the presence of a lateral mantle frill and the absence of longitudinal mantle folds, ventral mantle texture below the lateral frill, and patch and groove patterning. Additional traits could be used in combination to reliably characterize the novel morphotype. The genetic haplotypes of these octopuses were determined from nucleotide sequence data from two microsatellite loci and a portion of the OCDE gene. The GPO morphotype was identified with *E. dofleini* based on the match of body pattern traits to published descriptions of that species and the match of its genetic haplotype to published sequences of *E. dofleini*. Novel morphotype body patterns did not match descriptions of any species from the eastern north Pacific, while its genetic haplotype matched that of a recently sequenced undescribed octopus. The GPO and novel morphotypes are sister clades, and body pattern traits reliably identified individuals to morphotype and haplotype. Body pattern traits can be used in field identification of live octopuses allowing population assessments, by-catch frequency estimates, and other studies of both octopus types. We offer the common name of the frilled giant Pacific octopus for the novel morphotype, and based on genetic and morphological data suggest this clade is a new species of large Pacific octopus in the genus *Enteroctopus*. A full species description remains to be done.

Key words: *Enteroctopus*, taxonomy, cryptic diversity

Gleadall (1993) and Kaneko *et al.* (2011) have noted that the taxonomic status of large octopuses of the north Pacific is in need of clarification. Several factors make taxonomic study of these octopuses challenging, including poor definition of characters used in taxonomy (e.g. Norman and Hochberg 2005, Allcock *et al.* 2008), the absence of hard body parts and poor preservation of some soft-tissue characters in museum specimens (Roper and Sweeney 1983), and published descriptions and voucher collections comprised of a mixture of species (Pickford 1964, Hochberg and Fields 1980). The largest and most familiar of the large north Pacific octopuses, and the species with the greatest geographic range, is *Enteroctopus dofleini* (Wülker 1910), the giant Pacific octopus. This is a well-studied octopus species (e.g. Mottet 1975, Hartwick 1983, Hartwick and Barriga 1997, Scheel 2002) although basic taxonomic and life-history questions remain. For example, since its description (Wülker 1910, or possibly Hoyle 1885); eight different names have been applied to the species (Gleadall 2014). Taxonomic review of the large-ligula octopuses of the region has led to the suggestion that additional species recognized as belonging to the genus-*Octopus* Cuvier, 1797 are more correctly classified as *E. dofleini* congeners (Gleadall 1993, Kaneko *et al.* 2011). Subspecies of *E. dofleini* have been proposed (see Pickford 1964) but were not widely recognized (Gleadall 1993, Hochberg 1998), and Hochberg and Fields (1980) considered it likely the species comprised a complex with cryptic diversity.

The life history of *Enteroctopus dofleini* has been well-studied for an octopus, and is typical of many coastal octopus species. They live three to five years, and have a planktonic paralarvae stage that can last up to 90 days (Hartwick 1983, Hartwick and Barriga 1997). *Enteroctopus dofleini* are found throughout the North Pacific Rim from California north along the Alaska coast and west to Japan (Hartwick 1983, Jorgensen 2009). They occupy benthic habitats as sub-adults from the intertidal (Scheel 2002) to depths of possibly 1000m (Hochberg 1998) where they are benthic predators primarily of crabs and mollusks (Hartwick *et al.* 1981, Scheel and Anderson 2012). In the Gulf of Alaska, *E. dofleini* mature at weights of 10–20 kg (Conrath and Conners 2014). In near-shore environments off Hokkaido Japan, *E. dofleini* makes twice-annual onshore-offshore migrations in response to temperature fluctuations (Rigby and Sakurai 2005); however no evidence exists that such migration occur in the Alaskan or other eastern Pacific waters (Hartwick *et al.* 1984, Mather *et al.* 1985, Scheel and Bisson 2012).

A complete body pattern description for *Enteroctopus dofleini* has yet to be published (but see Anderson *et al.* 2003). Some of the characters used to distinguish this species include large size (> 0.5 kg or dorsal mantle length 10–35 cm), web

extending to the arm tips, longitudinal skin folds on the mantle (rather than tubercles), the presence of one large papillae over the eye, and a single frontal white spot below and between the eyes (rather than two or none). *Enteroctopus dofleini* also lack a lateral mantle ridge, lack three papillae under the eye ("eyelashes"), and lack ocelli (Hochberg and Fields 1980, Anderson 2006, Jorgensen 2009). Large size is commonly used to identify *E. dofleini*, however, Jorgensen (2009) reports that eye papillae and mantle texture characteristics are present in small post-settlement octopuses (3 cm mantle length). One of the authors (DS) has informally noted longitudinal skin folds, eye papillae, and a single frontal white spot in individuals at sizes under 100 g (unpublished data).

Recent population genetic studies of *Enteroctopus dofleini* have found two distinct clades in eastern Pacific waters (Toussaint *et al.* 2012b, Barry *et al.* 2013). These genetic studies focused on population properties and not taxonomic issues, and therefore neither study collected whole-animal voucher specimens nor examined the morphology or appearance of live animals. Hollenbeck and Scheel (2012) speculated that the two clades differed in body pattern and general appearance, however that report was based on an incomplete review of only two individuals. Body patterns are increasingly an important character in cephalopod taxonomy (Hanlon 1988, Roper and Hochberg 1988, Leite and Mather 2008), but these are best studied in living specimens. This study examined live specimens of octopuses from Prince William Sound, Alaska. We examined two genetic clades to test the hypotheses that there are two morphotypes that differ in body pattern characters, and that the morphotypes identify with the two clades with different genetic traits.

MATERIALS AND METHODS

Specimen and sample collection

We collected live octopuses on the Alaska Department of Fish and Game annual Spot shrimp (*Pandalus platyceros*) survey in Prince William Sound (PWS) Alaska during October (2012 and 2013); and in the same area from a commercial shrimp vessel (April 2013). The annual surveys were conducted at eight sites in northern and western PWS. A single string of eleven baited kite-style shrimp pots (6" x 16" x 36", covered with black woven fabric) was fished from depths between 55 m and 185 m at each of four stations within each site (44 pots per site). Soak times averaged 20–22 h. We collected octopuses as by-catch incidental to the shrimp surveys or shrimp fishery. An additional two octopuses were included in sampling that had been previously collected in central Prince William Sound by low-tide beach walk (Scheel 2002). Octopuses were either held alive for transportation to Alaska Pacific University aquarium laboratory, or photographed and measured in an aquarium on board the research vessel and released. We recorded octopus sex and weight. Skin swabs for epithelial cells were collected with 30 strokes from a cotton swab on the dorsal mantle and stored in a buffer solution until DNA extraction (Hollenbeck *et al.* 2017) and each animal was also biopsied for additional genetic material. After all live animal work was completed, select octopuses (Table 1) were euthanized via gradual freshwater drip, fixed in formalin (Roper and Sweeney 1983), preserved in anhydrous ethanol and deposited with the California Academy of Sciences (see Results). Limitations on the number of large specimens the California Academy could accept prevented us from depositing more animals.

Image collection

Photographic images were collected intermittently between 1–14 days post-capture, when the octopus was active or displaying different patterns. Most octopuses were photographed in the Alaska Pacific University aquarium lab in an acrylic aquarium 1.5 x 1.2 x 0.56 m under a mixture of florescent and natural lighting combined with strobe flash. Natural substrate comprised of shell hash, gravel, and rock was available on the bottom. Four octopuses were only photographed immersed in seawater in a white cooler without natural substrate using a submerged GoPro camera. These four individuals were photographed aboard the boat prior to release. In this study, mantle and eye texture, frontal white spots, and other body pattern traits tabulated were displayed by octopuses in either visual environment.

Body pattern traits

Body patterning traits were scored from photographs for each individual. We only scored each trait as present if visible in at least one photograph, and as absent only if not displayed in at least one photograph with the potential to capture the trait in question. The sample size of octopuses for any trait may be lower than the total number of octopuses studied: when a given trait was not visible in any photograph of a particular individual, that octopus was not counted toward the sample size for that trait. For example, if all photographs of a single octopus were taken when its skin texture was smooth, we could not score papillae character, and the octopus was not included in the sample size for that trait. Alternatively, if papillae or lateral mantle folds were displayed on the dorsal mantle in some photographs, but were not present in the same photographs on the ventral mantle, then the trait of "ventral mantle papillae or lateral skin folds" was scored as not present for that octopus. An octopus scored as not displaying a given trait was included in the sample total for that trait.

Traits scored were classified as follows, Lateral mantle frill comprised of a semi-continuous line of broad merged

Table 1. Octopuses sampled for genetic lineage and body patterning. All listed individuals were by-catch from shrimp pot fishing (except for Dena and Calamity) and all were genetically typed (except Calamity). Three were not photographed (underlined) so the sample size for body pattern was *Enteroctopus dofleini* (GPO) haplotype N = 14 and *cryptic* genetic clade N = 6 (genetically characterized in Toussaint et al. 2012b). Specimens with voucher IDs listed were accepted by the California Academy of Sciences Invertebrate Zoology (CASIZ) collection. For heterozygote individuals, both of the microsatellite allele sizes or OCDE alleles are indicated; otherwise the individual was homozygous. See Hollenbeck *et al.* 2017, Table 3 for OCDE allele variable nucleotide positions and details of genetic work.

Octopus ID	Genetic Clade	Edo μA 126	Edo μA 129	OCDE alleles	Morphotype	Weight (kg)	Sex	Voucher ID CASIZ
Dena	GPO	NA	145/153	OCDE1	GPO	0.86	F	
Calamity	GPO				GPO	1.19	F	
BBS13	GPO	126	149/153	OCDE1	GPO	1.52	F	
BRBGS13	GPO	126	145/153	OCDE1	GPO	1.82	F	
BRGF12	GPO	126	153	OCDE1	GPO	2.36	F	
GBRBF12	GPO	126	145	OCDE3	GPO	2.92	F	190942
RBGRF13	GPO	126	141/153	OCDE1	GPO	3.15	F	
RBRF13	GPO	126	139/147	OCDE1/4	GPO	3.55	F	
RBRS13	GPO	126	147	OCDE1	GPO	5.19	F	
GRBS13	GPO	126	153	OCDE1	GPO	6.62	F	
RBR3S13	GPO	126	145/147	OCDE1	GPO	1.62	M	
GBRBF13	GPO	126	145/155	OCDE1	GPO	2.90	M	
BRGS13	GPO	126	145/153	OCDE1	GPO	4.11	M	
GRBF13	GPO	126	153	OCDE1	GPO	5.45	M	
BGF13	GPO	126	145/153	OCDE1	GPO	5.55	M	
BS13	GPO	126	147	OCDE1	GPO	8.8	M	
SSF13	*cryptic*	110	127/133	OCDE2	novel	0.85	F	
BRGF13	*cryptic*	110	127/133	OCDE2	novel	2.15	F	193484
LGF13	*cryptic*	110	127/133	OCDE2	novel	0.25	M	
RBGRS13	*cryptic*	110	127	OCDE2	novel	0.42	M	193483
RGRF12	*cryptic*	110	127/133	OCDE2	novel	0.51	M	192393
BBF13	*cryptic*	110	127/133	OCDE2	novel	0.65	M	
RBRF12	*cryptic*	110	133	OCDE2	novel	0.82	M	190941

papillae forming a frill or flap along the mid-lateral mantle (Fig. 1A); White head bar across the top of the head from eye to eye; Light eye speckle (Fig. 2A); Character of the frontal white spot(s) as two spots (unmerged, separate and distinct, Fig. 2A) versus compound (*e.g.*, lobed, Fig. 2B) or simple (round or oval, Fig. 2C); Character of the primary papillae above the eye as long and thin (elongated flat and oar-like, Fig. 1C label 'lt') versus wide and fat (wide at the base and tapering like a cone, Fig. 1D), as branched (forked, Fig. 1C label 'br') versus simple (Fig. 1D), or as compound (with minor papillae at its base); Longitudinal skin folds on the mantle (Fig. 1B) (sensu Jorgensen 2009); Skin folds or papillae on the ventral mantle (Fig. 1B, contrast with Fig. 1A svm); Patch and groove (Fig. 3B, C) (sensu Packard and Hochberg 1977); Three papillae under the eye (Fig. 2C '3ey') (sensu Jorgensen 2009); Dorsal white spots; Webbing the length of the arms to tips (sensu Jorgensen 2009); and Dark eye bar (sensu Mather and Alupay 2016). Traits were scored a "0" if absent and a "1" if present.

Rapid lineage determination using microsatellite genotypes

Nucleotide sequence data from two microsatellite loci (Toussaint *et al.* 2012a, Toussaint *et al.* 2012b) were used to genetically type specimens as one of two haplotypes: either giant Pacific octopuses (the common lineage, identified with *Enteroctopus dofleini* in Toussaint *et al.* 2012b) or the rarer lineage, *cryptic* (Toussaint *et al.* 2012b). Hereafter, use of the term *cryptic* will be restricted to refer to this haplotype. *Cryptic* was also referred to as Clade II in Toussaint *et al.* (2012b). Microsatellite EdoμA129 appears diagnostic for *Enteroctopus dofleini* with allele sizes from 139–159, and for the *cryptic* with allele sizes 127–133 (Toussaint *et al.* 2012b), while microsatellite EdoμA106 appears fixed for allele 126 in *E. dofleini* but for allele 110 in the *cryptic* lineage (see Results, below). Rapid lineage determination from microsatellites was made within a few days post-capture, and independently of designation of morphotype based on body pattern traits.

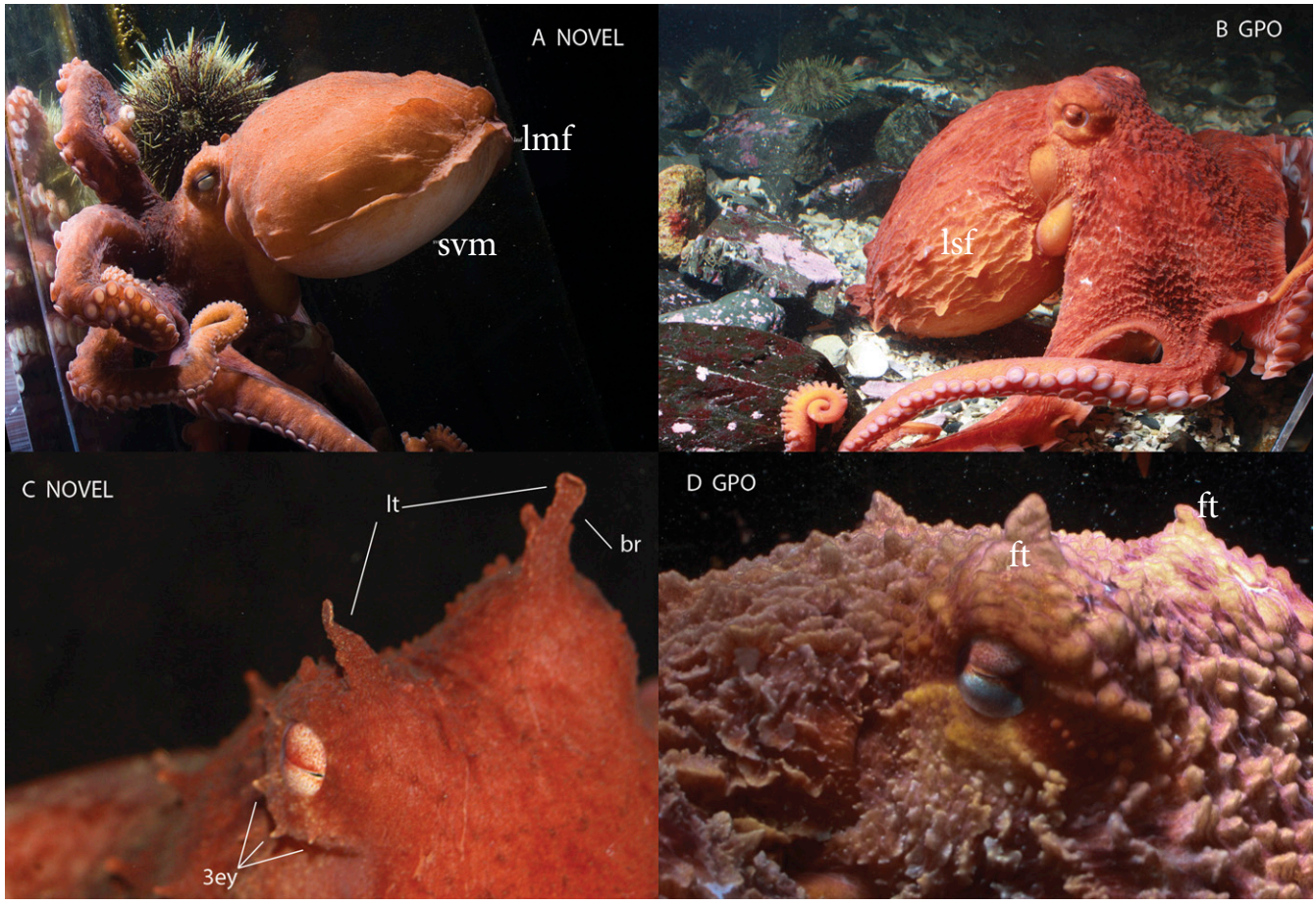

Figure 1. Mantle texture (top) and eye papillae (bottom) of the novel morphotype (left) and GPO morphotype (right), see Methods for additional details. A, Lateral mantle frill lmf, smooth texture on ventral mantle without longitudinal skin folds svm. B, Longitudinal skin folds lsf on dorsal, lateral and ventral mantle with no lateral frill. C, Eye papillae: primary papillae long, thin, and flat lt; branched br; and three minor papillae under the eye 3ey. D, Primary papillae over the eye fat and tapering ft, and lacking distinct papillae under the eye. Photos by DS.

Lineage membership was later confirmed with nucleotide sequences from a 612 bp fragment of the nuclear octopine dehydrogenase (OCDE) gene (Toussaint *et al.* 2012b) to corroborate the rapid microsatellite determination. OCDE appears to be fixed for allele OCDE2 in the *cryptic* clade, and is polymorphic for alleles OCDE1, OCDE3 and OCDE4 in *Enteroctopus dofleini* (Toussaint *et al.* 2012b). There is complete concordance between the *cryptic* lineage OCDE2 allele, EdoμA106 allele 110 and EdoμA129 allele sizes 127–133 (see Results, below).

Morphotype and Statistical analyses

Octopuses were assigned to two hypothesized morphotypes on the basis of a single published mantle texture trait of *Enteroctopus dofleini*. We then ask what if any other body pattern traits distinguish these morphotypes. Those displaying longitudinal skin folds on the dorsal mantle were assigned to the GPO morphotype (Hochberg 1998, Anderson 2006, Jorgensen 2009); those lacking longitudinal mantle folds were assigned to a novel morphotype. Hereafter, use of the phrase "novel morphotype" will be restricted to refer to this morphotype (lacking longitudinal folds), without reference to haplotype. Each octopus was assigned to one morphotype or the other on first observation post-capture, and prior to rapid lineage determination using microsatellites. Thus assignment to morphotype was independent of determination of genetic clade or analysis of other characters beyond longitudinal mantle folds.

After traits were scored, we identified clustering within the body pattern trait data. We calculated approximately unbiased bootstrap probabilities for dendogram branches based on Euclidean distance using the Ward cluster method of the "pvclust" command in the "pvclust" library of R (version 3.0.2). To eliminate missing values in the bootstrap

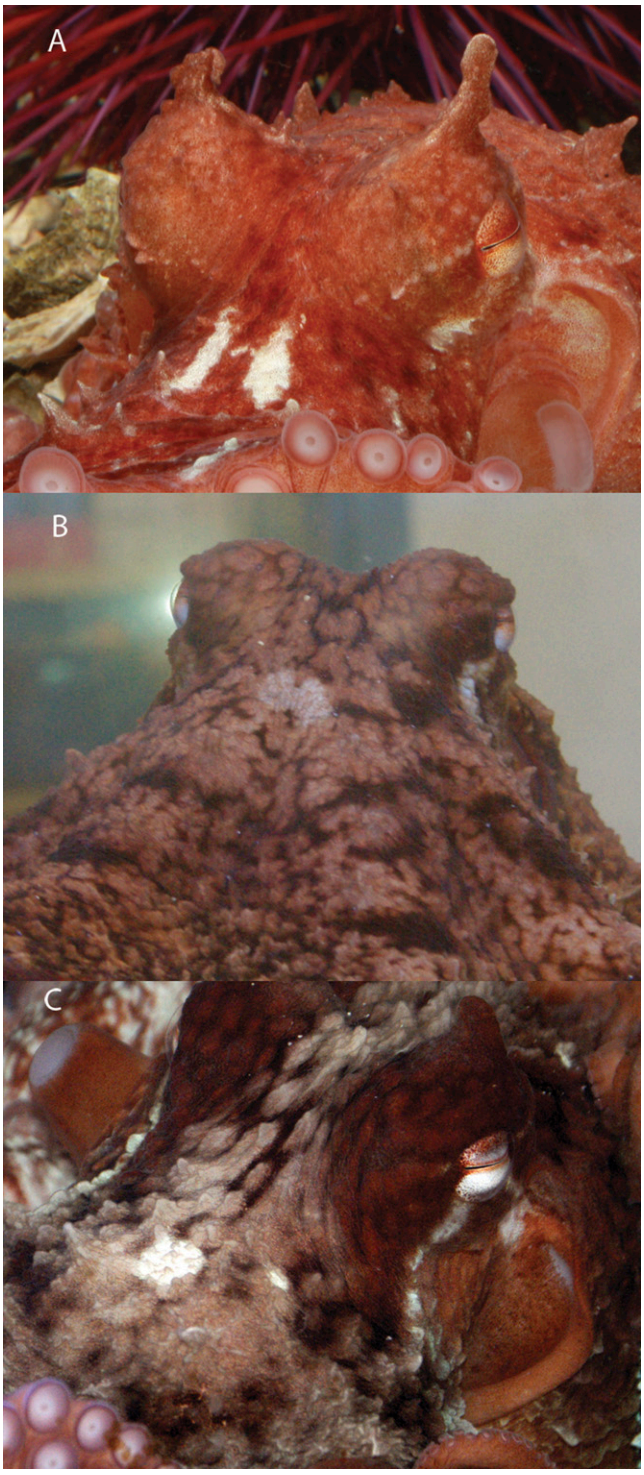

Figure 2. Frontal white spots, see Methods for additional details. On the novel morphotype, A, Two distinct simple spots. Photo by DS. On the GPO morphotype B, Compound spot, and C, Simple spot, each on different octopuses. Photos by NH.

analysis, we used a reduced dataset comprised of those six trait variables missing the fewest octopuses (patch and groove, lateral mantle frill, ventral mantle texture, longitudinal mantle folds, character of eye papillae as thin or fat, compound eye papillae).

RESULTS

Octopuses were captured as by-catch from eight sites sampled each October by the shrimp survey in western Prince William Sound, although we obtained octopus by-catch in each year from only a portion of these. Octopus capture rate was 2.95 per 100 pots pulled (N = 440 pots and 13 octopuses across both years). Additionally we obtained eight octopuses from commercial shrimp fishermen (April 2013) from an unknown number of pots. An additional two octopuses previously kept in the aquarium lab were also included in data collection. Both of these were photographed for body pattern traits, but only one was assessed for genetic lineage. Of 21 octopuses captured as by-catch in pots, N = 14 (66.6%) were genetic *Enteroctopus dofleini* and N = 7 (33.3)% were the *cryptic* clade (Table 1), based both on rapid lineage determination using microsatellite genotypes, and OCDE gene sequence. Maximum body mass for *E. dofleini* in this sample was 8.8 kg and for *cryptic* was 2.15 kg. These sizes likely represent sub-adults of *E. dofleini*. However, the novel morphotype octopus SSF13 (Table 1), a 0.85 kg female, made an abortive spawning attempt in captivity, possibly indicating a smaller size at maturity for this morphotype. She emitted a small mass of ruptured egg capsules (chorions) and attached stalks, as well as attaching four intact eggs by stalks to the aquarium wall. Eggs and ruptured chorions were approximately 6 mm long.

Shared components

We tabulated body pattern display components and a few gross morphological traits from photographs of N = 20 individuals (Table 1) Of 23 octopuses total, three were not photographed. Octopuses typically had in common webbing extending to the tips of the arms (one animal not scored), and head narrower than mantle (N = 20). In addition, all octopuses photographed with mixed light and dark components in their body patterns displayed two dorsal white spots if the dorsal mantle was photographed (N = 14). However, several individuals were only scored in a dark red body pattern in which it was not possible to assess the presence of white spots anywhere on the body. Dark eye bar was displayed by N = 19 of 20 photographed animals in at least one image. Finally, there were 11 males among the 23 captured animals, all identified by the presence of a ligula on the third right arm (Table 1).

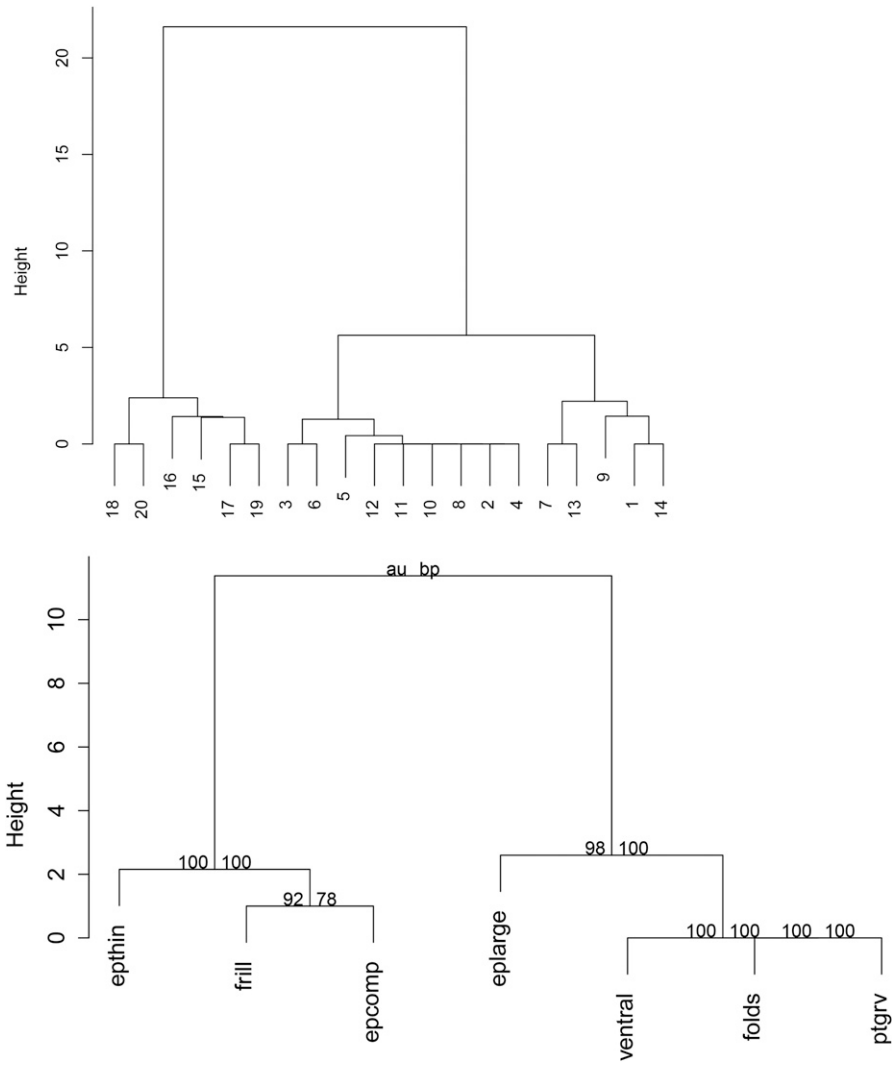

Figure 3. Dendograms of individual octopuses (top) and traits (bottom) showing clusters derived from body pattern characteristics. Octopus individuals 1–14 were GPO morphotype and genetic *Enteroctopus dofleini*, and individuals 15–20 were novel morphotype and the *cryptic* genetic clade. Branch numbers indicate the 'au'=approximately unbiased probability (left number) and 'bp'=bootstrap probability (right number). Traits included were ptgrv, patch and groove; folds, longitudinal mantle folds; ventral, papillae or folds on the ventral mantle; eplarge, large eye papillae; epcomp, compound eye papillae; frill, lateral mantle frill; and epthin, thin eye papillae.

Morphotype components

We characterized octopuses according to body patterns into two morphotypes (Table 2): in the first (GPO morphotype), octopuses displayed longitudinal folds on the dorsal, lateral and portions of the ventral mantle. In the second group (the novel morphotype), octopuses lacked longitudinal mantle folds anywhere on the mantle, displayed a lateral mantle frill, and although displaying papillae above the lateral mantle frill on the dorsal mantle, lacked any mantle texture below the lateral mantle frill on the ventral mantle. Dorsal mantle folds or papillae were not always displayed by any octopus, but when they were displayed, a smooth ventral mantle on the novel morphotype (Fig. 1A) contrasted with ventral longitudinal mantle folds displayed by the GPO morphotype (Fig. 1B). Eye papillae components of body patterns were commonly associated with either the GPO morphotype or the novel morphotype, but not exclusively except in combinations (see below).

Cluster analysis of a reduced dataset (eliminating missing values, N = 19 octopuses and N = 7 traits) also identified the same two groups of individuals (Fig. 3, top) with 100% bootstrap probability based on the presence of patch and groove, longitudinal mantle folds, and ventral mantle texture in the GPO morphotype and on the lateral mantle frill and thin eye papillae in the novel morphotype (Fig. 3, bottom, $P < 0.05$).

Textural components

The trait of longitudinal mantle folds was displayed on dorsal, lateral and ventral mantle in all individuals of the GPO morphotype (N = 14, Fig. 1B). All novel morphotype animals (N = 6 photographed) lack the longitudinal mantle skin folds anywhere on the mantle and lacked textural folds or papillae entirely on the ventral mantle which appeared smooth in all body patterns (Fig. 1A). Additionally in the novel morphotype, the ventral and dorsal sides of the mantle were divided by a lateral mantle frill formed by merged papillae along the longitudinal line of the mantle (Fig. 1A). This frill was visible although reduced in expression even when body texture elsewhere including the rest of the mantle was smooth and unpapillated. No frill was ever visible in any GPO morphotype individual.

Eye papillae components

All six novel morphotype animals had long thin eye papillae with primarily parallel sides along their length (Fig. 1C). Only three (23% of N = 13) of the GPO morphotype individuals had long thin eye papillae like this and the remaining

Table 2. The proportion of two octopus morphotypes scored from photos displaying a body pattern trait (and the sample size of scored individuals), grouped according to the morphotype for which the trait is most characteristic. As traits were not always continuously visible (displayed), failure to record the trait in a photograph does not mean the trait could not ever be displayed by an individual.

	Haplotype		
Trait	***E. dofleini***	***cryptic***	**Morphotype**
Lateral mantle frill	0.00 (14)	1.00 (6)	Novel
Eye papillae, compound	0.00 (13)	0.83 (6)	
Three papillae under eye	0.00 (12)	0.83 (6)	
Frontal white spots, two distinct	0.14 (7)	1.00 (6)	
Eye papillae, long and thin	0.23 (13)	1.00 (6)	
White head bar, eye to eye	0.00 (1)	1.00 (4)	
Light eye speckle	0.08 (12)	1.00 (6)	
Eye papillae, branched	0.00 (10)	0.33 (6)	
Dorsal white spots	1.00 (8)	1.00 (6)	Shared
Web extends to tip of arms	1.00 (13)	1.00 (6)	
Dark eye bar	1.00 (13)	1.00 (6)	
Head narrower than mantle	1.00 (14)	1.00 (6)	
Frontal white spots, single compound	0.57 (7)	0.00 (6)	GPO
Frontal white spots, single simple	0.28 (7)	0.00 (6)	
Eye papillae, wide and fat	0.77 (13)	0.00 (6)	
Ventral mantle papillae or skin folds	1.00 (14)	0.00 (6)	
Patch and groove	1.00 (14)	0.00 (6)	
Longitudinal skin folds	1.00 (14)	0.00 (6)	

N = 10 (77%) had wide, fat papillae above the eye. Additionally all novel morphotype animals had some kind of complex eye papillae traits (Table 2): either three 'eyelash' papillae under the eye, or branched or compound papillae above the eye (Fig. 1C). Although individually, no single one of these complex eye papillae traits were found in all novel morphotype individuals, no GPO morphotype octopus displayed any of these three traits, while all novel morphotype octopuses displayed at least one, and 4 (66% of N = 6) displayed more than one.

Chromatic components

Of those GPO morphotype octopuses that we were able to score for frontal white spots, most (Table 1. N = 6 of 7, 85%) displayed either compound (Fig. 2B) or simple single frontal white spots (Fig. 2C). One GPO morphotype individual appeared to have two distinctly separate spots. In contrast, all novel morphotype individuals displayed two distinct intense frontal white spots (Fig. 2A). Novel morphotype white spots were never merged or singular and were often elongated.

A light colored eye speckle (as many light colored small spots) and a white head bar were displayed by 100% of novel morphotype individuals (Table 2). These traits were not typical in GPO morphotype, although we were unable to score them for all individuals in the sample (no more than 8% of scored GPO morphotype octopuses, Table 2). Patch and groove reticulation (Fig. 2B) was displayed by 100% (N = 14) of GPO morphotype animals, and was visible in different body patterns of this morphotype. Patch and groove reticulation was not observed in any novel morphotype animals (N = 6) for any body pattern.

Genetic lineages of morphotypes

To ensure that morphology group assignment by researchers was unbiased by the genetic results, each individual was assigned to a morphotype group after genetic sampling but before genetic results were available. Of those octopuses for which we had body pattern photographs and genetic lineage, N = 13 (100%) GPO morphotype octopuses (longitudinal mantle skin folds displayed on dorsal, lateral and ventral mantle but no lateral frill) were typed genetically as *Enteroctopus dofleini*, while N = 6 (100%) novel morphotype individuals (lateral mantle frill but no longitudinal mantle skin folds) were typed genetically as *cryptic* lineage octopuses (Table 1).

Novel morphotype – cryptic lineage octopuses

Without exception, the octopuses described above were obtained from western Prince William Sound pot-fishing and (two *Enteroctopus dofleini* only) central PWS intertidal beach walk. We opportunistically examined photographs of two further octopuses from other geographic areas. From the Bering Sea, we examined photographs of an octopus captured from Zhemchug Canyon as fisheries by-catch and donated to the Alaska Sea Life Center. We also examined photographs of an octopus taken by a SCUBA diver during a night dive at 20 m depth near Juneau, AK. Both octopuses were of the novel morphotype based on the absence of longitudinal mantle folds and the presence of a lateral mantle frill. In the Bering Sea individual only, it was also possible to recognize from the photographs the novel morphotype traits of papillae on the dorsal mantle but an absence of texture on the ventral mantle, two distinct frontal white spots, long thin papillae above the eye, and three 'eyelash' papillae below the eye.

The captured octopus from the Bering Sea also possessed the OCDE2 allele and EdoμA129 allele size of 133, both of which occur in the *cryptic* lineage but not in *Enteroctopus dofleini*. Microsatellite locus EdoμA129 did not amplify in this sample. We therefore tentatively describe the range of the novel morphotype (and therefore the *cryptic* genetic lineage) as at least extending from the Bering Sea to Juneau, Alaska.

DISCUSSION

Our analyses of morphological characteristics of body patterning revealed two types of octopus in Prince William Sound Alaska. GPO morphotype octopuses were distinguished without exception from the novel morphotype by the presence of three traits: longitudinal folds in mantle texture, ventral mantle texture, and patch and groove patterning (Figs. 1, 3), and the absence of one trait (lateral mantle frill, Table 1 and Fig. 1). Additional, more variable, traits that distinguished the morphotypes were frontal white spots (Fig. 2), white head bar, and complex eye papillae components (Figs. 1, 3) including three papillae under eye, long thin eye papillae, and compound or branched papillae over the eye. Within each morphotype, there was variation in the character of papillae around the eye (Table 2). Body pattern around the eye also comprised a major component in overall body pattern variation in *Octopus cyanea* Gray, 1849 (Benolkin 2016). Frontal white spots also varied within morphotypes (*e.g.*, Fig. 2 GPO morphotype) although we did not attempt to further characterize the variation.

Although body pattern characters visible on living octopuses do not preserve well and therefore are not always used by taxonomists working with museum specimens (Norman and Hochberg 2005), field keys to distinguish live octopuses and taxonomists working from live animals (*e.g.*, Leite and Mather 2008) typically rely on body patterns. In North Pacific waters, octopus size is also important and coastal reddish octopuses over 0.5 kg often are assumed to be *Enteroctopus dofleini* (the giant Pacific octopus) based on size (*e.g.*, Jorgensen 2009, Reuter *et al.* 2010). The traits of the GPO morphotype match the published descriptions of *E. dofleini*, including most notably size >500 g, the presence of longitudinal mantle folds extending to the lateral and ventral mantle, the large papillae over the eye, patch and groove patterning, and a single frontal white spot. Thus none of the traits tabulated here for the GPO morphotype distinguished these octopuses from *E. dofleini*. All individuals of this morphotype were also genetically typed as *E. dofleini*, and we therefore identify the GPO morphotype octopuses as this species.

The suite of body pattern traits identified here as the novel morphotype was found only in octopuses belonging to the *Clade II* genetic lineage (*sensu* Toussaint *et al.* 2012b), itself identified with the genetic haplotype *cryptic* (Hollenbeck *et al.* 2017). However, the body patterns of the novel morphotype do not match the description of any known species, although there are similarities between novel morphotype traits and those found in other species.

Benthoctopus leioderma (Berry 1911) is captured from waters deeper than 500 m and displays a lateral mantle ridge that might be confused with the lateral mantle frill of the novel morphotype. However, *B. leioderma* body texture is otherwise smooth, it has unusually large eyes, and its known range does not include Prince William Sound. The mantle texture and character of the lateral mantle ridge of *B. leioderma* distinguish this species from the novel morphotype.

Octopus rubescens (Berry 1953) (the red octopus) occurs along coastal waters from California to British Columbia, and displays three minor papillae under the eye and two distinct frontal white spots (Anderson 2006, Jorgensen 2009) that might be confused with the three papillae under the eye and two frontal white spots of the novel morphotype. However, *O. rubescens* is also characterized by small size (< 500 g) and pebbly mantle texture, and this octopus displays transverse bands across the mantle not seen in either morphotype described here. The maximum body size, mantle texture, and lack of transverse bands all distinguished the novel morphotype from *O. rubescens*, along with the presence in the novel morphotype octopuses of the lateral mantle frill which is absent in *O. rubescens*. The range of *O. rubescens* reported by some authors has included "Alaska" (*e.g.*, Sosa *et al.* 1995), and maybe at least portions of Prince William Sound (*e.g.*, to 60° N, Hochberg 1997, to central Alaska, Cosgrove and McDaniel 2009). However, in 20 years (1995 to 2016) of intertidal, SCUBA, pot, and submersible surveys for octopuses in Prince William Sound conducted by DS, no *O. rubescens* have been found (Scheel 2002, Scheel *et al.* 2007, Scheel 2015). Jorgensen (2009) indicated a northernmost extent of its range at the southeast Alaska border with British Columbia. Given the common traits of two frontal white spots and three minor papillae under the eye, it is possible that small novel morphotype specimens from Alaskan waters may have been mistaken for *O. rubescens*.

Enteroctopus dofleini and the *cryptic* haplotype octopus are sister clades, based on the re-creation in Toussaint *et al.* (2012b) of the maximum-parsimony tree presented by Kaneko *et al.* (2011) using nucleotide sequence data from the mitochondrial gene cytochrome c oxidase subunit I (COI). *Enteroctopus dofleini* and the novel morphotype also shared a number of body pattern traits, including characteristics of relative head size, web extent, dark eye bars, and patterns of dorsal mantle white spots. Toussaint *et al.* (2012b) suggest the *cryptic* clade is a congener of *E. dofleini*.

However, the two types are not the same. Genetically, the two clades were separated by 3–4% COI sequence divergence, while within-clade divergence was less than 1%. The clades were separated across mtDNA and nuclear genes as well as microsatellite loci; and they were reciprocally monophyletic (Toussaint *et al.* 2012b). In this report, we establish that the two clades are also clearly and easily distinguished by body pattern traits displayed by living animals.

The mantle folds vary among the species of *Enteroctopus* in typical coloration and their relationship to other aspects of skin texture (*e.g.*, Villanueva *et al.* 1991). Body pattern descriptions for this group are generally poor and revision has been called for in the north Pacific membership of the genus (*e.g.*, Gleadall 1993, Kaneko *et al.* 2011). Based on its large size and other shared characters with *E. dofleini* (Table 2), as well as its genetic status as a sister clade to *E. dofleini* (Toussaint *et al.* 2012b), the novel morphotype is a new species in *Enteroctopus*. We offer the common name of the frilled giant Pacific octopus for the novel morphotype, based on the lateral mantle frill and large body size. Live octopuses were successfully assigned to lineages based on easily observed body patterning traits. A complete taxonomic description remains to be done.

Prince William Sound is in the middle of the known range of *Enteroctopus dofleini* (California along the Pacific rim to Japan, Pickford 1964, Hartwick 1983, Jorgensen 2009). We found that the range of the frilled giant Pacific octopus similarly extends at least from southeast Alaska to the Bering Sea. Octopus population structure from southeast and south-central Alaska based on mitochondrial sequence data (COI) also suggested the presence of an undocumented species within the *E. dofleini* complex (Barry *et al.* 2013), possibly equivalent to the frilled giant Pacific octopus of this report and the *cryptic* (Clade II) haplotype of Toussaint *et al.* (2012b) and Hollenbeck *et al.* (2017).

Both octopus types occur as by-catch in shrimp pots at least in Prince William Sound. Large octopuses (previously considered *Enteroctopus dofleini*) are a significant portion of by-catch in Alaska, yet it is unknown what portion of the catch of large octopuses actually is of the frilled giant Pacific octopus (Reuter *et al.* 2010). From pots in our study, *E. dofleini* occurred as 67% of our total catch while frilled giant Pacific octopuses occurred as 33% of the total (Table I). Frilled giant Pacific octopuses reach at least 2.15 kg (Table 1), which should be considered a minimum estimate given the small number of animals weighed (Table 1). It remains to be determined over what range octopus by-catch, even of large octopuses, is a mixture of *E. dofleini* and other species. In western Prince William Sound, at least in this shrimp survey, frilled giant Pacific octopus comprised a large portion of octopus by-catch in 2012 and 2013. Additional collection in 2014 (data not presented) were comprised of 74% (of N = 19) frilled giant Pacific octopuses, identified by both morphotype using traits described in this report, and by haplotype using rapid microsatellite lineage determination. Field identification of live octopuses based on traits described here will allow assessment of the true frequency of each type occurring in by-catch from Alaska waters anywhere these two octopus types co-occur.

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