



Effects of fixation and long-term preservation on finned octopods (Cephalopoda: Cirrata)

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Received: 20 February 2023 / Accepted: 2 August 2023 / Published online: 27 September 2023
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Abstract

Among mollusks, the octopods stand out due to the almost entire absence of a stabilizing shell, potentially rendering these organisms susceptible to deformation caused by fixation and preservation. Such artifacts have previously been shown to occur especially in specimens of the deep-sea taxon Cirrata, the finned octopods. As an adaptation to their extreme habitat, many cirrates are composed of (semi-)gelatinous tissues, which are particularly prone to distortion, shrinkage, and deformation following fixation and preservation. Using one or more species from each of the eight currently recognized cirrate genera, the present study provides pre- and post-fixation color imagery of entire specimens as well as measurements of taxonomically relevant characters. These novel data illustrate the sometimes drastic effects that short- and long-term chemical treatment entails for this cephalopod taxon. Both structural and color changes may occur when finned octopods are fixed and preserved using routine protocols. However, shrinkage does not occur uniformly in all taxonomically relevant structures. The consequences of such a treatment for species descriptions and identification are discussed and existing anaesthetization, fixation and preservation protocols are summarized.

Keywords Octopus · Collection · Formalin · Ethanol · Shrinkage · Protocol

Introduction

To preserve extant zoological material for future reference as well as further study, collected specimens are conventionally treated using a variety of chemical protocols. These lead to the fixation of tissues in order to prevent decay *post-mortem* and additionally permit preservation of the specimen by preventing microbial degradation during long-term storage. While fixation and preservation form the basis for creation and maintenance of natural history collections, all chemicals routinely employed are known to have one or more side effects (Russell 1963; Steedman 1976; Lincoln and Sheals 1979; Williams and Van Syoc 2007; Simmons 2014). Due to an almost entire absence of stabilizing hard parts, soft-bodied organisms such as cephalopods (Mollusca: Cephalopoda) are particularly prone to deformation, distortion, and shrinkage

caused by chemical treatment for fixation and preservation purposes (Boyde and Barber 1969; Cohen 1976; Roper and Sweeney 1983; Andriquetto and Haimovici 1988; Voss and Pearcy 1990; Voight 1991; O'Shea 1997, 1999; Gleadall et al. 2010; Jereb et al. 2014). As a rule of thumb, those cephalopod specimens with more gelatinous tissues suffer increased structural alteration during fixation and preservation than their more muscular peers (Robson 1932; Voight 2001).

Semi-gelatinous to gelatinous species are particularly common among deep-sea cephalopod taxa such as the finned octopods (Octopoda: Cirrata), sister group to the much better-known, predominantly shallow-water finless octopods (Octopoda: Incirrata). In fact, cirrates are the deepest known cephalopods, occurring down to a bathymetric depth of at least 6957 m (Jamieson and Vecchione 2020). In cirrates, life in an extreme habitat has led to a number of structural adaptations, e.g. the presence of elastic, fluid-rich, gelatinous tissues that are supposed to aid in maintaining neutral buoyancy (Aldred et al. 1978, 1983; Roper and Voss 1983). However, these structural properties render cirrates difficult candidates for fixation and long-term preservation (Collins et al. 2001; Collins and Villanueva 2006). For example, a typical artifact known to occur in cirrate museum specimens

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is that the eyes and fins appear relatively much larger following fixation and preservation due to significant shrinkage of the surrounding mantle tissues (Young and Vecchione 2016). Given that morphometric characters and relational measurements still constitute important, if not essential arguments for cephalopod species description and identification, deformations may have significant taxonomic consequences.

Although the often quite drastic effects of fixation and preservation on cirrate specimens have been known for a long time (e.g. Eschricht 1836; Reinhardt and Prosch 1846; Meyer 1906; Ebersbach 1915), no study has so far systematically documented the degree of deformation, distortion, or shrinkage found in fixed material of this group of cephalopods. By taking advantage of the fact that digital color imagery of freshly caught cirrate specimens has become increasingly available over the past 2 decades (e.g. Piatkowski and Diekmann 2005; Allcock 2014; Shea et al. 2018; Golikov et al. 2020; Ziegler and Sagorny 2021; Verhoeff 2022), we here present a systematic visual and morphometric overview of adult specimens incorporating at least one species per extant cirrate genus. In addition, comparable imagery for selected cirrate juveniles as well as a direct comparison between a cirrate and an incirrate deep-sea specimen treated identically and documented over known time intervals are presented. The combined data provide an overview of structural and color changes exhibited by cirrates chemically treated from about 1 month to almost 20 years. The present article permits a better evaluation of the suitability of certain morphometric and relational characters for cirrate species description and identification. In addition, the study provides an overview of established protocols for the anaesthetization, fixation and preservation of finned octopods.

Materials and methods

Specimens

Selection of specimens for this study was conducted based on several minimum requirements, in particular the availability of (1) high-resolution color photography pre- and post-fixation, (2) scaled imagery, (3) photographs with comparable specimen orientation, and (4) documented archival history. In addition, adult as well as juvenile specimens were selected to cover a large spectrum of ontogenetic stages. The resulting dataset comprises specimens mentioned in previous publications as well as unpublished material. However, all specimens incorporated into the present study (Table 1) are deposited in one of the following museum collections for future reference: BMNH=Natural History Museum, London, United Kingdom; LACM=Natural History Museum of Los Angeles County, Los Angeles, CA, USA; MNHNCL=Museo Nacional de Historia Natural de Chile, Santiago de Chile, Chile; NIWA=National Institute of Water and Atmospheric Research, Wellington,

New Zealand; NMNS=National Museum of Natural Science, Taichung City, Taiwan; NMV=Museums Victoria, Melbourne, Victoria, Australia; USNM=Smithsonian National Museum of Natural History, Washington, DC, USA; YPM=Peabody Museum of Natural History, New Haven, CT, USA; ZMB=Museum für Naturkunde, Berlin, Germany; ZMH=Museum der Natur, Hamburg, Germany.

Photography

The photographs shown here were made over the past 4 decades using a broad range of classical and digital cameras with different lighting setups. Wherever possible, specimens were placed into liquid-filled containers and photographed with a uniform background. Where applicable, the photographer of a given image is mentioned in the figure legend. Due to the heterogeneity of the imagery shown here, color standardization using color charts was not implemented. Therefore, numerical color comparisons are not possible using the present dataset.

Measurements and indices

Where possible, morphological characters were measured on the physical specimen itself using calipers or rulers both pre- and post-fixation. In addition, standard measurements were obtained from the literature. However, in multiple cases, measurement data were gathered based on calibrated imagery. These virtual measurements were conducted using the free software Fiji/ImageJ 1.54b (Schindelin et al. 2012). Designation of body measurements and indices follows Collins (2003) and Verhoeff and O'Shea (2022): AL=arm length (here: of longest arm), ALI=arm length index, CLI=cirrus length index, ED=eye diameter, EDI=eye diameter index, FL=fin length, FLI=fin length index, FS=fin span, FuL=funnel length, FuLI=funnel length index, FW=fin width, FWI=fin width index, HW=head width, HWI=head width index, MCL=maximum cirrus length, ML=mantle length (here: dorsal ML, where applicable), MSD=maximum sucker diameter, MW=mantle width, MWI=mantle width index, PA=pallial aperture gape, PAI=pallial aperture gape index, SDI=sucker diameter index, TL=total length. The entire set of morphometric data is provided in Table 1.

Results

Effects of fixation and long-term preservation on adult cirrate specimens

Cirroteuthidae Keferstein, 1866

A medium-sized female specimen of *Cirrothauma magna* (Hoyle, 1855) with 190 mm ML was collected from the

Table 1 List of species and specimens incorporated into the present study showing sex, fixation protocol, date of documentation as well as standard measurements (in mm) with respective shrinkage (in %), and indices (in %)

Species	Specimen	Sex	Fixation	Date (d/m/y)	TL	ML	MW	HW	FL	FW	FS	ED	FuL	PA	AL	MCL	MSD	MWI	HWI	FLI	FWI	EDI	FuLI	PAI	ALI	CLI	SDI
<i>Cirrothauma magna</i>	USNM 1502928	F	10% FO, 50% IA	04/07/2009	815	190	171	182	102	65	370	52	38	33	628	65	6	90	96	54	64	27	20	17	331	34	3
				28/02/2023	625	128	117	178	105	54	288	51	35	30	448	48	6	91	139	82	51	40	27	23	350	38	5
<i>Cirrotaeuthis cf. muelleri</i>	NMV F245713	F	Shrinkage 25/05/2017	08/12/2020	169	56	37	42	50	22	130	11	13	93	66	75	89	44	20	23	166						
				03/03/2008	444	66	50	64	27	18	88	23	338	76	97	41	67	35	512								
<i>Stauroteuthis gilchristi</i>	NIWA 41297	U	FO, ET	07/12/2022	174	33	21	25	17	10	55	9	127	64	76	52	59	27	385								
				Shrinkage 23/04/1995	446	185	203	215	94	44	245	28	44	259	4	110	116	51	47	15	24	140	2				
<i>Lueteuthis shuishi</i>	NMNS 002157-00088	F	10% FO, 70% ET	2000-2001	268	88	98	96	63	32	220	17	26	172	3	111	109	72	51	19	30	195	3				
				Shrinkage 27/05/2017	372	99	85	117	45	31	174	35	48	252	86	118	45	69	35	48	25	224					
<i>Grimpotaeuthis abyssicola</i>	NMV F245714	M	10% FO, 70% ET	08/12/2020	245	50	46	65	47	19	140	28	21	19	133	92	130	94	40	56	42	38	266				
				Shrinkage 23/06/2010	201	73	72	78	30	20	129	15	17	19	40	7	2	99	107	41	67	21	23	26	10	3	
<i>Grimpotaeuthis angularis</i>	NIWA 68993	F	5% FO, 70% ET	03/04/2020	152	56	46	46	38	18	109	15	14	13	6	2	82	82	68	47	27	25	23	11	4		
				Shrinkage 05/07/2016	290	95	61	69	69	35	199	17	21	17	3	3	64	73	73	51	18	22	18	3	3		
<i>Grimpotaeuthis imperator</i>	ZMB MOLL 240160	M	10% FO, 70% ET	29/11/2017	283	81	45	63	61	32	167	17	18	15	3	3	56	78	75	52	21	22	19	4	4		
				Shrinkage 30/07/2000	427	128	72	84	70	31	201	15	14	12	0	0	56	66	55	44	12	221					
<i>Grimpotaeuthis wuelkeri</i>	ZMH 12244	U	4% FO, 70% ET	01/04/2019	220	55	32	37	44	17	117	14	156	58	67	80	39	25	284								

Table 1 (continued)

Species	Specimen	Sex	Fixation	Date (d/m/y)	TL	ML	MW	HW	FL	FW	FS	ED	FuL	PA	AL	MCL	MSD	MWI	HWI	FLI	FWI	EDI	FuLI	PAI	ALI	CLI	SDI
<i>Cryptoteuthis breviparachiata</i>	BMNH 20040614	F		Shrinkage	48	57	56	56	37	45	42	7			45			161	168	34	93						222
				25/04/2001	148	41	66	69	14	13	93																
<i>Cirrotopus cf. glacialis</i>	YPM IZ 049350	U		FO, 80% IMS	04/02/2021	99	32	21	27	16	10	55			66			66	84	50	63						206
				Shrinkage	04/03/2009	295	85	89	81	64	41	210	16					27			105	95	75	64	19		
<i>Opisthotentacles bruanii</i>	MNHNCL 300139	F		FO, 70% ET	05/12/2017	230	71	64	75	60	32	183	15		140			90	106	85	53	21				197	
				Shrinkage	04/09/2008	216	64	76	98	26	22	103	27				15			119	153	41	85	42			
<i>Opisthotentacles chathamensis</i>	NIWA 68989	U		96% ET	25/11/2022	92	24	36	38	14	8	64	13		53			150	158	58	57	54				221	
				Shrinkage	19/06/2010	210	55	67	99	28	20	128	21				144			122	180	51	71	38			
<i>Opisthotentacles plato</i>	NMV F245704	U		FO, ET	07/12/2022	177	32	38	55	30	13	94	14		128			119	172	94	43	44				400	
				Shrinkage	22/05/2017	262	104	115	135	32	33	171	25				11			111	130	31	103	24			
<i>Opisthotentacles robsoni</i>	NIWA 68987	U		10% FO, 70% ET	28/11/2022	184	61	58	64	42	28	132	25		106			95	105	69	67	41				174	
				Shrinkage	19/06/2010	329	78	105	148	34	26	165	30				33			135	190	44	76	38			
<i>Grimpotentacles sp.</i>	USNM 1460905	H		FO, 70% ET	07/12/2022	257	56	74	85	28	23	128	24		183			132	152	50	82	43				327	
				Shrinkage	31/08/2005	32	13	10	11	15	8	41	2.7				16			77	85	115	53				
<i>Grimpotentacles sp.</i>	USNM 1460906	H		4% FO, 70% ET	29/10/2015	30	12	10	10	14	6	35	2.5		15			83	83	117	43					125	
				Shrinkage	21/08/2005	21	10	10	8	12	6	31	2	3.2			11			100	80	120	50	20			
<i>Grimpotentacles sp.</i>	USNM 1460906	H		4% FO, 70% ET	02/11/2015	16	10	10	8	11	4	29	2	3	6			100	80	110	36	20	30			60	
				Shrinkage		24	0	0	0	0	8	33	6	0	6	45	0	45									

Table 1 (continued)

Species	Specimen	Sex	Fixation	Date (d/m/y)	TL	ML	MW	HW	FL	FW	FS	ED	FuL	PA	AL	MCL	MSD	MWI	HWI	FLI	FWI	EDI	FuLI	PAI	ALI	CLI	SDI	
<i>Opisthotenthis</i> sp.	LACM DJSCO 4111	J		12/11/2009	22	8	10	12	4	3	16	4			13		125	150	50	50	75	50					163	
			95% ET	21/08/2017	19	7	8	9	4	3	18	4			7		114	129	57	57	75	57					100	
<i>Grimpoenthis imperator</i>			Shrinkage		14	13	20	25	0	0	-13	0		46														
			05/07/2016	290	95		69			35		17															18	
			4% FO	19/04/2017	287	88		67		34		17																19
			Shrinkage		1	7		3		3		0																
			4% FO	22/09/2017	287	88		67		34		17																19
			70% ET	29/11/2017	283	81		63		32		17																21
<i>Musoctopus</i> sp.			70% ET	24/04/2019	281	80		60		27		17															21	
			Shrinkage		3	16		13		23		0																
			27/07/2016	179	48		37					14															29	
			4% FO	19/04/2017	178	47		37				14																30
			Shrinkage		1	2		0				0																
			4% FO	22/09/2017	177	46		36				14																30
			70% ET	29/11/2017	176	46		36			14																30	
			Shrinkage		2	4		3			0																	
			70% ET	24/04/2019	176	45		36				14															31	
			Shrinkage		2	6		3				0																

ET ethanol, F female, FO formalin, H hatching, IA isopropyl alcohol, IMS industrial methylated spirit, J juvenile, M male, U unsexed—please see the Materials and methods section for all other abbreviations

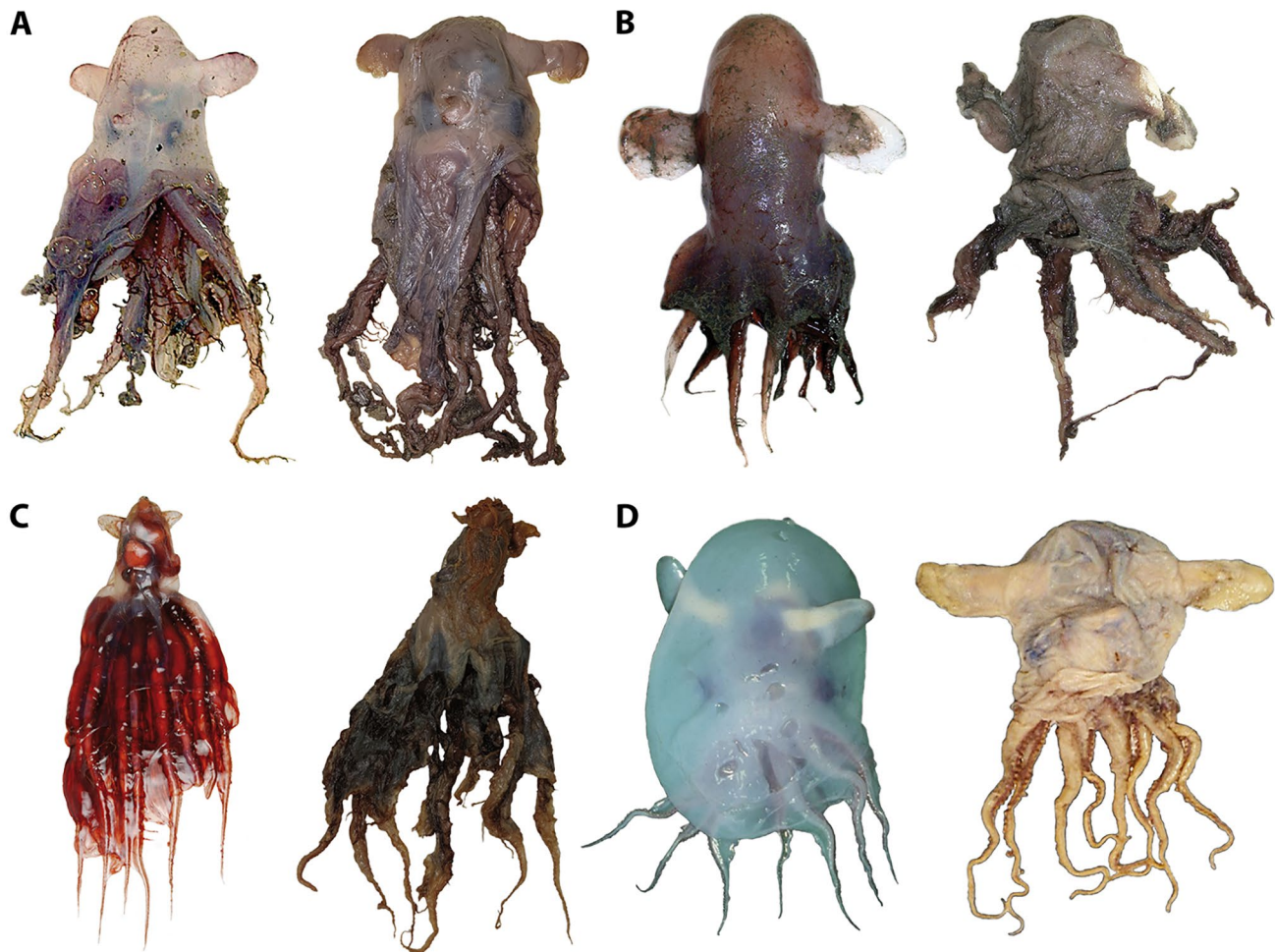


Fig. 1 Effects of the fixation and long-term preservation on selected adult cirrate specimens of the genera *Cirrothauma*, *Cirroteuthis*, *Stauroteuthis*, and *Luteuthis*—left image depicts fresh and right image shows preserved specimen. **A** *Cirrothauma magna*, USNM 1502928, formalin-fixed and preserved in isopropyl alcohol, interval 13 years and 293 days, 190 mm vs. 128 mm ML, ventral views (courtesy Michael Vecchione). **B** *Cirroteuthis* cf. *muelleri*, NMV F245713, formalin-fixed and ethanol-preserved, interval 3 years and 197 days, 116 mm vs. 56 mm ML, dorsal views (courtesy Karen Gowlett-Hol-

mes and Tristan J. Verhoeff, respectively). **C** *Stauroteuthis gilchristi*, NIWA 41297, formalin-fixed and ethanol-preserved, interval 14 years and 278 days, 66 mm vs. 33 mm ML, dorsal views (courtesy Darren Stevens and Sadie Mills, respectively). **D** *Luteuthis shuishi*, NMNS 002157–00088, formalin-fixed and ethanol-preserved, interval 5–6 years, 185 mm vs. 88 mm ML, dorsal views (courtesy Ping-Ho Ho and Wen-Sung Chung, respectively). Note that color in these images has not been standardized

Mid-Atlantic Ridge in the Atlantic Ocean at 2995–3071 m depth (Richards and Vecchione 2020). The specimen was photographed following its collection on 04 July 2009 (Fig. 1A, left), subsequently placed in 10% formalin for fixation and later in 50% isopropyl alcohol for preservation. 13 years and 293 days later (28 February 2023), the museum specimen (USNM 1502928) was retrieved from the preservative and photographed again (Fig. 1A, right). Fixation and preservation resulted in significant shrinkage of the posterior-most part of the mantle and a tightening of the primary web around the arms. However, no significant changes in coloration were observed, although the violet hue of the arms seemed to have faded to a uniform pinkish

coloration of the entire body. Shrinkage was most pronounced for ML (33%), MW (32%), and AL (29%), while TL shrank by 23% (Table 1).

The small female specimen of *Cirroteuthis* cf. *muelleri* Eschricht, 1836 with 116 mm ML shown here (Fig. 1B, left) was collected off East Gippsland in the Tasman Sea at 2338–2581 m depth (Verhoeff 2022). This specimen was photographed fresh on 25 May 2017 and was then placed in 10% formalin for fixation, followed by preservation in 70% ethanol in the museum collection (NMV F245713). After 3 years and 197 days, the specimen was photographed again on 08 December 2020 (Fig. 1B, right). Treatment with fixative and preservative resulted in

substantial deformation of the specimen's body, in particular of the primary web, the fins, and the posterior-most part of the mantle. The overall color of the specimen changed from red-violet to grey-brown and the semi-transparent aspect in certain parts of the body was almost entirely lost. The most pronounced shrinkage was observed for FW (54%) and ML as well as MW (both: 52%), TL shrank by 42% (Table 1).

A medium-sized unsexed specimen of *Stauroteuthis gilchristi* (Robson, 1924) with 66 mm ML was collected in the Scott seamounts area of the Southern Ocean at 855–879 m depth. The specimen was photographed following its capture on 03 March 2008 (Fig. 1C, left). It was then placed in formalin for fixation and later transferred to ethanol for long-term preservation. After 14 years and 278 days in the collection, the museum specimen (NIWA 41297) was again photographed on 07 December 2022. Chemical treatment resulted in significant changes to the specimen's aspect with almost all body parts affected, in particular primary web, arms, fins, and mantle. The color of the specimen changed from a vivid, semi-transparent dark-red to a dull, dark-brown hue with patches of a dirty-red color in the mantle area (Fig. 1C, right). Shrinkage was most pronounced for AL (62%) and amounted to 61% for HW, ED, and TL each (Table 1).

Grimpoteuthidae O'Shea, 1999

The single known specimen of *Luteuthis shuishi* O'Shea & Lu, 2002 was a female collected off Pratas Islands in the South China Sea at 754–767 m depth (O'Shea and Lu 2002). This particularly gelatinous specimen with 185 mm ML was photographed on 23 April 1995 while still fresh (Fig. 1D, left). The organism was fixed using 10% formalin and transferred to 70% ethanol after several days (Lu 2010). About 5–6 years later, the museum specimen (NMNS 002157–00088) was photographed again (Fig. 1D, right). Changes to the appearance of the mantle of this individual are most obvious: due to the reduction of the mantle's size, the fins appear significantly larger in relation to the body. Because of chemical treatment, the aspect of the specimen changed from a whitish semi-transparency to a non-transparent beige color. Shrinkage in this specimen was most pronounced for HW (55%) as well as ML and MW (both: 52%), while shrinkage of TL was 40% (Table 1).

A medium-sized specimen of *Grimpoteuthis abyssicola* O'Shea, 1999 with 99 mm ML was obtained off Bermagui in the Tasman Sea at 2687–2821 m depth (Verhoeff and O'Shea 2022). This male was photographed fresh on 27 May 2017 (Fig. 2A, left), subsequently was placed in 10% buffered formalin and later transferred to 70% ethanol. After 3 years and 195 days in solution, the museum specimen (NMV F245714) was again photographed on 08 December 2020

(Fig. 2A, right). Chemical treatment of this semi-gelatinous individual resulted in significant deformation of the mantle and fins in particular. Because of fixation and preservation, semi-transparency of the fins, mantle, and arms was lost, and the specimen showed a dull-brown aspect. Shrinkage in this specimen was most obvious for FuL (56%), but ML (49%) and MW (46%) were also significantly affected. In turn, TL shrank by 34% (Table 1).

A medium-sized female specimen of the recently described species *Grimpoteuthis angularis* Verhoeff & O'Shea, 2022 was collected on the Chatham Rise in the Pacific Ocean at 627–628 m depth (Verhoeff and O'Shea 2022). This specimen with 73 mm ML was photographed fresh on 23 June 2010 (Fig. 2B, left), prior to its fixation in 5% formalin and subsequent transfer to 70% ethanol for preservation. On 03 April 2020, i.e. after 9 years and 285 days, the specimen (NIWA 68993) was removed from its jar for additional photography (Fig. 2B, right). Deformation was quite pronounced, with distortion of arms, mantle, and fins particularly visible. Chemical treatment led to an almost complete loss of transparency of the mantle and fins—the specimen showed a dull-white aspect. Externally visible morphological characters most affected by shrinkage were HW (41%), MW (36%), and PA (32%). Due to the significant shrinkage of the mantle, FL increased by 27%, while TL was reduced by 24% (Table 1).

The single known specimen of the recently described *Grimpoteuthis imperator* Ziegler & Sagorny, 2021 was collected on Tenji Seamount in the Pacific Ocean at 3912–4417 m depth (Werner et al. 2016; Ziegler 2021; Ziegler and Sagorny 2021). This medium-sized male with 95 mm ML was photographed fresh on 05 July 2016 (Fig. 2C, left) before being placed in a fixative composed of 10% buffered formalin and later transferred to 70% ethanol—please see the cirrate-incirrate comparison below for the precise sequence of events. The museum specimen (ZMB MOLL 240160) was removed from the preservative for photography after 1 year and 147 days on 29 November 2017 (Fig. 2C, right). Changes to the overall aspect were notable for the mantle and primary web in particular: the somewhat inflated mantle appeared reduced in size, while the primary web had retracted. The overall color of the animal had changed from white-purple to white-beige, but with semi-transparency largely preserved. The most prominent shrinkage occurred in MW (26%), FS (16%), and ML (15%), while TL was reduced by 2% (Table 1).

A single, unsexed specimen of *Grimpoteuthis wuelkeri* (Grimpe, 1920) with 128 mm ML was collected in the Angola Basin area of the Atlantic Ocean at or above 5430 m depth (Piatkowski and Diekmann 2005). The specimen was photographed upon arrival on deck on 30 July 2000 (Fig. 2D, left) and was then fixed using 4% buffered formalin. On land, the museum specimen (ZMH 12244)

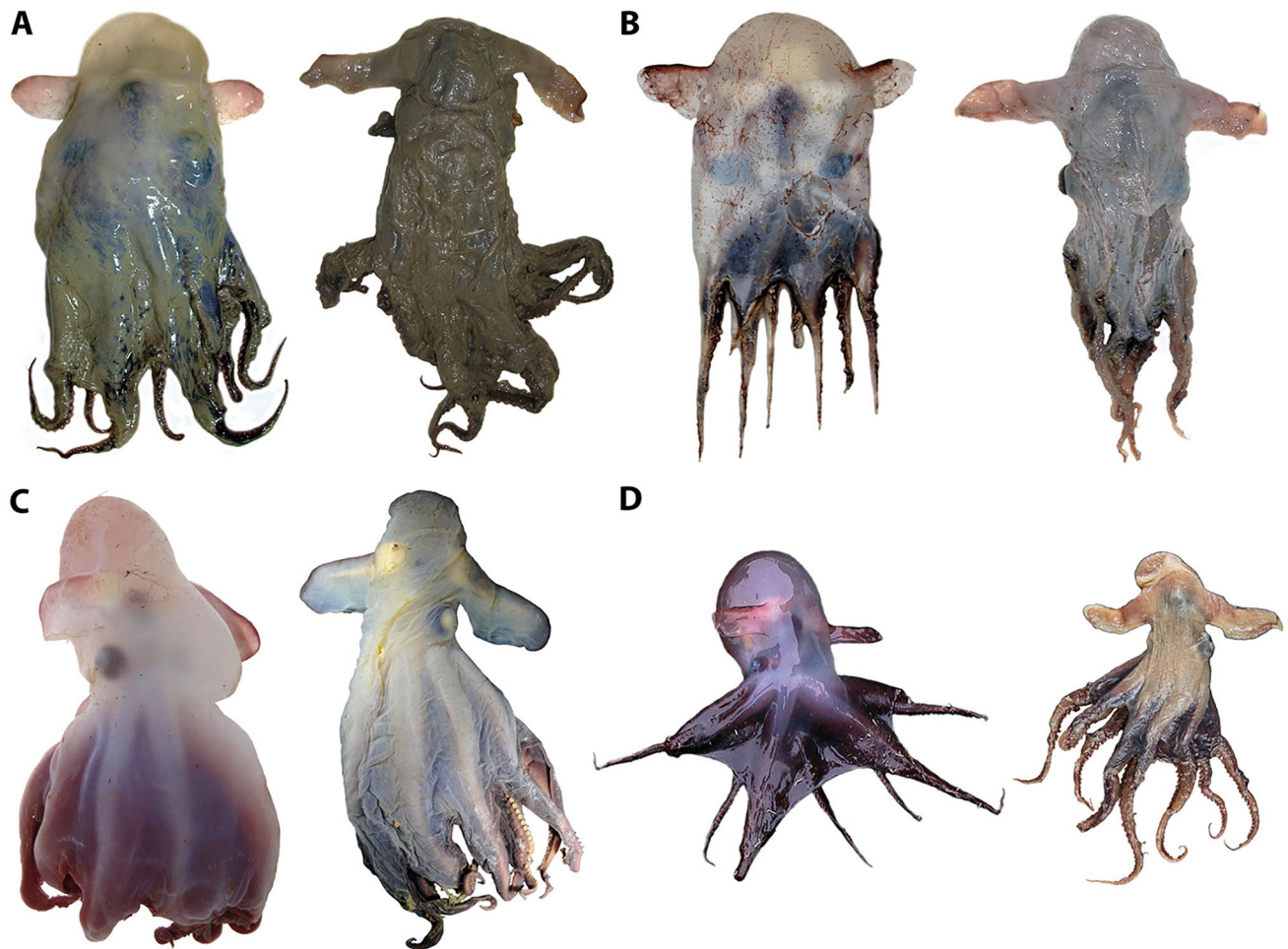


Fig. 2 Effects of the fixation and long-term preservation on selected adult cirrate specimens of the genus *Grimptoteuthis*—left image depicts fresh and right image shows preserved specimen. **A** *Grimptoteuthis abyssicola*, NMV F245714, formalin-fixed and ethanol-preserved, interval 3 years and 195 days, 99 mm vs. 50 mm ML, dorsal views (courtesy Karen Gowlett-Holmes and Tristan J. Verhoeff, respectively). **B** *Grimptoteuthis angularis*, NIWA 68993, formalin-fixed and ethanol-preserved, interval 9 years and 285 days, 73 mm

vs. 56 mm ML, dorsal views (courtesy Darren Stevens and Tristan J. Verhoeff, respectively). **C** *Grimptoteuthis imperator*, ZMB MOLL 240160, formalin-fixed and ethanol-preserved, interval 1 year and 147 days, 95 mm vs. 81 mm ML, dorsal views. **D** *Grimptoteuthis wuelkeri*, ZMH 12244, formalin-fixed and ethanol-preserved, interval 18 years and 245 days, 128 mm vs. 55 mm ML, dorsal views (courtesy Michael Türkay and Bernhard Hausdorf, respectively). Note that color in these images has not been standardized

was transferred to 70% ethanol on 21 March 2001 and photographed again on 01 April 2019, i.e. 18 years and 245 days following its capture (Fig. 2D, right). The most obvious changes to the appearance of the organism following chemical treatment were reductions in the relative size of mantle, fins, and primary web. Due to the deformation of the mantle, the fins appeared much larger in the fixed specimen (Fig. 2D, right). The color of the mantle had changed from a vivid purple primary web and semi-transparent whitish hue of the mantle to a dull purple web and a beige, almost non-transparent aspect. Shrinkage was most pronounced for ML (57%) and MW as well as HW (both: 56%), while TL shrank by 48% (Table 1).

The single known specimen of *Cryptoteuthis brevibrachiata* Collins, 2004 was obtained from the Porcupine Seabight in the Atlantic Ocean at 2274–2300 m depth (Collins 2004). This medium-sized female with 41 mm ML was photographed fresh on 25 April 2001 (Fig. 3A, left), prior to its fixation in formalin and subsequent long-term preservation in 80% industrial methylated spirit for storage as a museum specimen (BMNH 20040614). After 19 years and 285 days following capture, the animal was photographed again on 04 February 2021 (Fig. 3A, right). Fixation and preservation led to pronounced changes in the overall aspect of the specimen: the arms were bent in an almost rectangular angle toward the dorsal mantle and the fins appeared relatively much larger than in the fresh specimen. The color

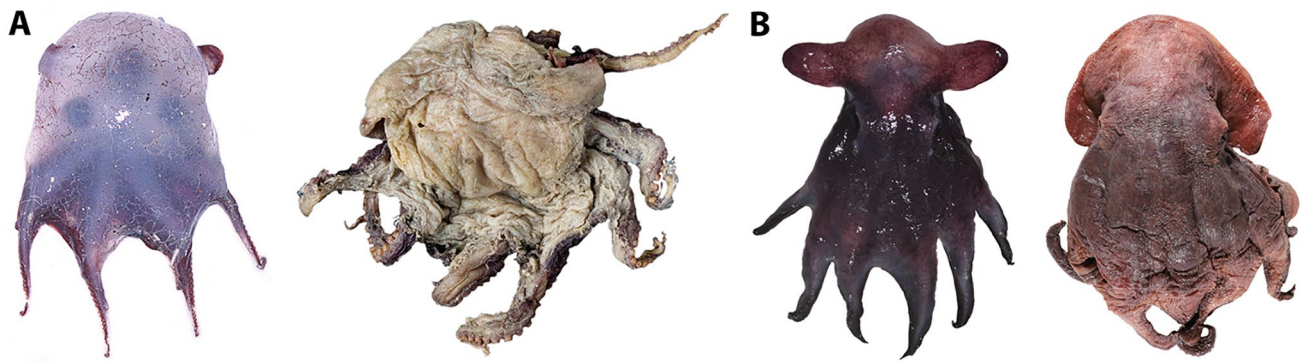


Fig. 3 Effects of the fixation and long-term preservation on selected adult cirrate specimens of the genera *Cryptoteuthis* and *Cirroctopus*—left image depicts fresh and right image shows preserved specimen. **A** *Cryptoteuthis brevibrachiata*, BMNH 20040614, formalin-fixed and preserved in industrial methylated spirit, interval 19 years and 285 days, 41 mm vs. 32 mm ML, dorsal views (courtesy Martin

A. Collins and Jon Ablett, respectively). **B** *Cirroctopus* cf. *glacialis*, YPM IZ 049350, formalin-fixed and ethanol-preserved, interval 8 years and 276 days, 85 mm vs. 71 mm ML, dorsal views (courtesy Eric A. Lazo-Wasem). Note that color in these images has not been standardized

had changed from light purple, semi-transparent to a dull-beige hue. Shrinkage was most obvious for MW (68%), HW (61%), and FS (41%). As in *G. abyssicola* and *G. angularis* (see above), shrinkage of the mantle resulted in an increase in FL (here: by 14%). TL was reduced by 33% (Table 1).

Cirroctopodidae Collins & Villanueva, 2006

An unsexed specimen tentatively assigned to *Cirroctopus* cf. *glacialis* (Robson, 1930) based on its place of origin was collected off the Antarctic Peninsula in the Southern Ocean at 627–628 m depth (Allcock 2014). The specimen with 85 mm ML was photographed fresh on 04 March 2009 prior to its fixation in formalin and subsequent preservation in 70% ethanol (Fig. 3B, left). The museum specimen (YPM IZ 049350) was photographed again on 05 December 2017, i.e. after 8 years and 276 days following capture. Due to the more muscular habitus of cirroctopodids in general (Vecchione et al. 1998), the deformation of this specimen was not as pronounced as in the more gelatinous cirrate species (Fig. 3B, right). However, the animal appeared more bent toward the ventral side, the arms were somewhat contracted against the upper body, and the fins no longer were oriented perpendicular to the mantle. The overall color scheme had changed from a vivid dark-purple to a dull, pinkish-brown. Shrinkage was less pronounced than in other cirrates, but was most obvious for MW (28%) as well as FW (22%) and ML (16%), while TL shrank by 22% (Table 1).

Opisthoteuthidae Verrill, 1896

A medium-sized female of *Opisthoteuthis bruuni* (Voss, 1982) with 64 mm ML was found off the Algarrobo coast in the Pacific Ocean at 512 m depth (Ibañez et al. 2011;

Pardo-Gandarillas et al. 2021). It was photographed fresh on 04 September 2008 (Fig. 4A, left) before being placed directly in 96% ethanol for fixation and long-term preservation. 14 years and 82 days later, the museum specimen (MNHCL 300139) was removed from its jar on 25 November 2022 and photographed again (Fig. 4A, right). The pictures illustrate the drastic changes in body structure and color that took place due to chemical treatment. Mantle, primary web, fin, and arm tissues contracted significantly, and the entire animal appeared compressed. The color of the specimen changed from a vivid, sprinkled, bright-red semi-transparency to a dull black-to-beige aspect without any transparent tissues. Shrinkage was most pronounced for FW (64%) and ML as well as AL (both: 63%). Correspondingly, shrinkage of TL was observed to be 57% (Table 1).

An almost globose, unsexed specimen of *Opisthoteuthis chathamensis* O’Shea, 1999 was collected on the Chatham Rise in the Pacific Ocean at 1176–1178 m depth. Following its capture on 19 June 2010, the animal with 55 mm ML was photographed while still fresh (Fig. 4B, left). Fixation and preservation most likely consisted of formalin and subsequent ethanol treatment of the museum specimen (NIWA 68989). On 07 December 2022, i.e. 12 years and 171 days later, the specimen was again photographed (Fig. 4B, right). The images document significant changes to primary web, mantle, and fin shape. The color of the initially not very transparent specimen changed from a vivid purple to a dull red-brown aspect. Shrinkage was most pronounced for HW (44%), MW (43%), and ML (42%), while TL shrank by 63% (Table 1).

The medium-sized, unsexed specimen of *Opisthoteuthis pluto* Berry, 1918 with 104 mm ML obtained from the Bass Strait in the Pacific Ocean at 2692–2760 m depth was photographed fresh on 22 May 2017 (Fig. 4C, left) and was then

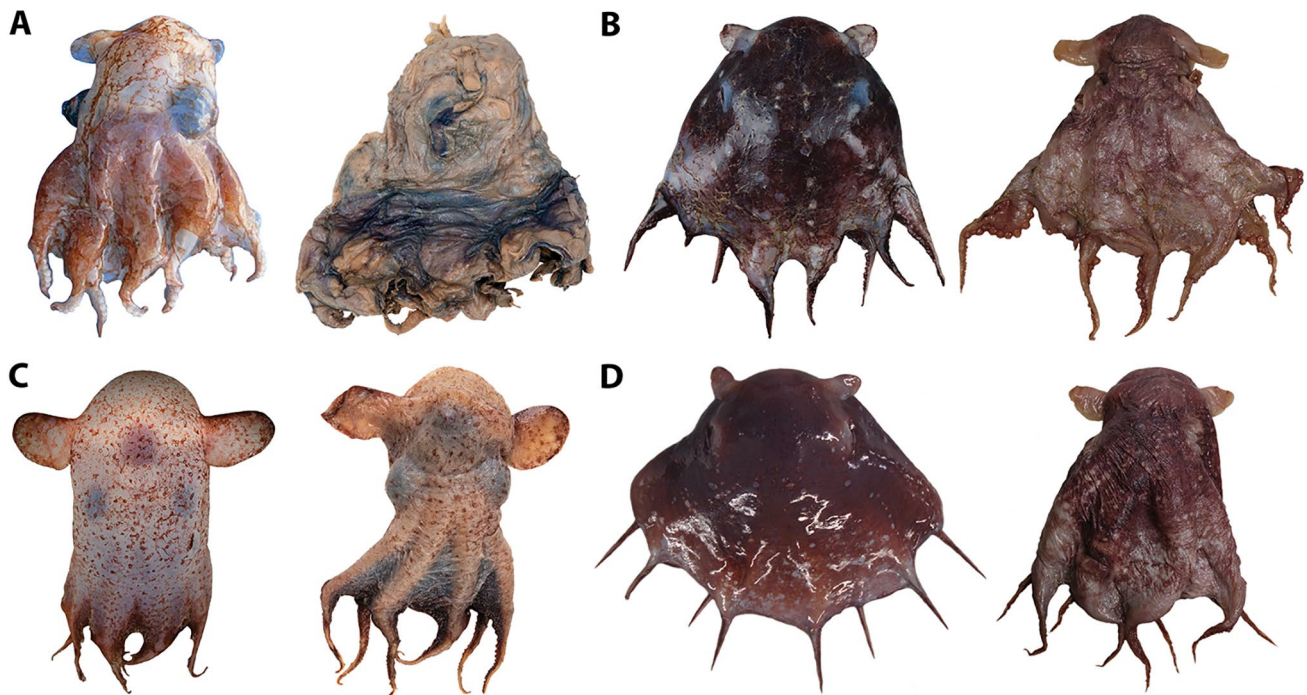


Fig. 4 Effects of fixation and long-term preservation on selected adult cirrates of the genus *Opisthoteuthis*—left image depicts fresh and right image shows preserved specimen. **A** *Opisthoteuthis bruuni*, MNHNCL 300139, ethanol-fixed and ethanol-preserved, interval 14 years and 82 days, 64 mm vs. 24 mm ML, dorsal and left lateral view (courtesy Diana Párraga and Catalina Amanda Merino Yun-nissi, respectively). **B** *Opisthoteuthis chathamensis*, NIWA 68989, formalin-fixed and ethanol-preserved, interval 12 years and 171 days, 55 mm vs. 32 mm ML, dorsal views (courtesy Darren Stevens and

Sadie Mills, respectively). **C** *Opisthoteuthis pluto*, NMV F245704, formalin-fixed and ethanol-preserved, interval 5 years and 190 days, 104 mm vs. 61 mm ML, dorsal views (courtesy Robert Zugaro and Julian K. Finn, respectively). **D** *Opisthoteuthis robsoni*, NIWA 68987, formalin-fixed and ethanol-preserved, interval 12 years and 171 days, 78 mm vs. 56 mm ML, dorsal views (courtesy Darren Stevens and Sadie Mills, respectively). Note that color in these images has not been standardized

fixed using 10% formalin and later preserved in 70% ethanol. After an interval of 5 years and 190 days, the museum specimen (NMV F245704) was once again photographed (28 November 2022). Immediately notable was the overall reduction in mantle size as well as the tightening of the primary web around the arms (Fig. 4C, right). In addition, the eyes appeared much larger in the fixed specimen. Surprisingly, the overall color scheme did not change much, although part of the semi-transparency had waned. Nonetheless, shrinkage was quite pronounced, most so for HW (53%), MW (50%), and ML (41%), while TL shrank by 30% (Table 1).

A small-sized, unsexed specimen of *Opisthoteuthis robsoni* O’Shea, 1999 with 78 mm ML was obtained from the Chatham Rise in the Pacific Ocean at 1,599–1,619 m depth. The animal was photographed fresh on 19 June 2010 (Fig. 4D, left) prior to a likely treatment using formalin and later 70% ethanol. After an interval of 12 years and 171 days, the museum specimen (NIWA 68987) was photographed on 07 December 2022 (Fig. 4D, right). Predominantly mantle and primary web tissues appeared to be affected by chemical treatment. The fins of the fixed specimen seemingly were larger compared to the fresh specimen. The color did not

change much, although the more vivid appearance of the fresh specimen was replaced by a rather dull hue. Shrinkage was most pronounced for HW (43%), MW (30%), and ML (28%), while TL shrank by 22% (Table 1).

Effects of fixation and long-term preservation on premature cirrate specimens

A single hatchling of *Grimpoteuthis* sp. emerged from a cirrate egg capsule following collection of its deep-sea octocoral host organism (Anthozoa: Octocorallia) on Kelvin Seamount in the Atlantic Ocean at 1965 m depth (Shea et al. 2018; Ziegler 2018, 2021; Ziegler et al. 2021; Ziegler and Miller 2024a). Immediately afterwards, the hatchling with 13 mm ML was photographed fresh on 31 August 2005 (Fig. 5A, left). Following its direct observation on-board ship, the cirrate juvenile specimen was fixed in 4% formalin and later transferred to 70% ethanol. 10 years and 59 days later, the museum specimen (USNM 1460905) was photographed again on 29 October 2015 (Fig. 5A, center and right). Chemical treatment resulted in deformation of the fins and the mantle tissues in particular. The color of the

Fig. 5 Effects of the fixation and long-term preservation on selected premature cirrate specimens of the genera *Grimpot euthis* and *Opisthot euthis*—left image depicts fresh and right images show preserved specimen. **A** *Grimpot euthis* sp., USNM 1460905, formalin-fixed and ethanol-preserved, interval 10 years and 59 days, 13 mm vs. 12 mm ML, dorsal and ventral views (courtesy Timothy M. Shank and Elizabeth K. Shea, respectively). **B** *Grimpot euthis* sp., USNM 1460906, formalin-fixed and ethanol-preserved, interval 10 years and 73 days, 10 mm vs. 10 mm ML, dorsal and ventral views (courtesy Timothy M. Shank and Elizabeth K. Shea, respectively). **C** *Opisthot euthis* sp., LACM DISCO 4111, ethanol-fixed and ethanol-preserved, interval 7 years and 282 days, 8 mm vs. 7 mm ML, dorsal and ventral views (courtesy Cheryl A. Brantley and Regina Wetzer, respectively). Note that color in these images has not been standardized



specimen changed from a vivid dark-red with semi-transparent fins to a dull beige with pronounced transparency of mantle and fins, likely caused by the almost entire loss of the original pigmentation (Fig. 5A, center and right). Shrinkage was most pronounced for FW and MSD (both: 25%) as well as FS (15%), while TL shrank by 6% (Tab. 1). It is noteworthy to mention that this premature hatchling was characterized by the presence of a large internal yolk sac (Shea et al. 2018; Ziegler et al. 2021), potentially restricting a more pronounced shrinkage of the surrounding body parts.

Another single, but less mature hatchling of *Grimpot euthis* sp. with 10 mm ML was obtained from the Corner Rise Seamounts in the Atlantic Ocean at 2068 m depth (Ziegler 2021; Ziegler et al. 2021; Ziegler and Miller 2024a). Following photography immediately after hatching on 21 August 2005 (Fig. 5B, left), the specimen was fixed using 4% formalin and then transferred to 70% ethanol for long-term preservation as a museum specimen (USNM 1460906). The fixed individual was photographed again after 10 years and 73 days on 02 November 2015 (Fig. 5B). All external organ systems were affected by deformation: arms, primary web, mantle, and fins. The color of the specimen changed from a vivid orange with semi-transparent fins and arms to a dull-beige aspect with pronounced transparency of mantle and fins, again likely caused

by the almost entire loss of the original pigmentation (Fig. 5B, center and right). Shrinkage was most obvious for AL and MSD (both: 45%) as well as FW (33%), while TL shrank by 24% (Table 1). As in the previous specimen, this premature hatchling was characterized by the presence of a large internal yolk sac (Ziegler et al. 2021).

An early juvenile of *Opisthot euthis* sp. was dredged off Palos Verdes in the Pacific Ocean at 458–459 m depth (Ziegler and Miller 2024b). Prior to its direct fixation in 95% ethanol, this individual with 8 mm ML was photographed fresh on 12 November 2009 (Fig. 5C, left). After 7 years and 282 days in the fixative, the museum specimen (LACM DISCO 4111) was once again photographed on 21 August 2017 (Fig. 5C, center and right). Fixation led to a significant degree of deformation in all body parts, in particular of the mantle tissues. The color of the specimen changed from a vivid orange-red to a dull beige with underlying reddish tones (Fig. 5C, center and right). Shrinkage was most pronounced for AL (46%), HW (25%), and MW (20%), while TL shrank by 14% (Table 1). The absence or presence of an internal yolk sac could not be ascertained.

Direct comparison between cirrate and incirrate adult specimens

One cirrate as well as several incirrate specimens were collected in 2016 during deep-sea dredging operations in the North Pacific Ocean. These specimens were all treated in the exact same way following capture (Werner et al. 2016). This provided the opportunity to use two of these specimens as reference material for a comparative study on the effects of fixation and preservation in specimens from each of the two octopod lineages.

The cirrate specimen was the above-mentioned male of the newly described species *G. imperator*. This specimen with 95 mm ML was collected and photographed fresh on 05 July 2016 (Fig. 6A, left). Its mantle was filled turgid, the fins were inflated, and mantle as well as funnel were covered by numerous red pigment spots. In addition, the ventral surface of primary web and arms as well as the posterior rim of the fins were dark red (Fig. 6A, left). Following photography, the

specimen was transferred into a drum filled with 5 l of 4% buffered formalin. After 9 months and 14 days in the fixative, the museum specimen (ZMB MOLL 240160) was removed from the drum for photography on 19 April 2017 (Fig. 6A, center left). Shrinkage was most pronounced for ML (7%), while TL shrank by 1% (Table 1). After another 5 months and 3 days in the fixative, the specimen was photographed again on 22 September 2017 (Fig. 6A, center)—no further shrinkage was observed. Following this round of photography, the specimen was then transferred to 70% ethanol for long-term preservation. After 2 months and 7 days in the preservative, the specimen was photographed again on 29 November 2017 (Fig. 6A, center right). Shrinkage as compared to the fresh specimen was most pronounced for ML (15%) as well as HW and FW (both: 9%), while TL shrank by 2%. Following another year and 146 days in the preservative, the cirrate was photographed for the last time on 24 April 2019 (Fig. 6A, right). Compared to the fresh specimen collected



Fig. 6 Comparison of the effects of an identical fixation and long-term preservation protocol on one adult cirrate and one adult incirrate specimen. **A** *Grimptoteuthis imperator*, ZMB MOLL 240160. Photographed fresh following collection on 05 July 2016 with 95 mm ML (left), formalin-fixed for 9 months and 14 days with 88 mm ML (center left) as well as for further 5 months and 3 days with 88 mm ML (center), then ethanol-preserved for 2 months and 7 days with 81 mm ML (center right) as well as an additional 1 year and 146 days

with 80 mm ML (right), right lateral views. **B** *Muusoctopus* sp., ZMB MOLL 240161. Photographed fresh following collection on 27 July 2016 with 48 mm ML (left), formalin-fixed for 8 months and 23 days with 47 mm ML (center left) as well as further 5 months and 3 days with 46 mm ML (center), then ethanol-preserved for 2 months and 7 days with 46 mm ML (center right) as well as an additional 1 year and 146 days with 45 mm ML (right), dorsal views. Note that color in these images has not been standardized

and photographed 2 years and 293 days earlier, shrinkage was most pronounced for FW (23%), ML (16%), and HW (13%), while TL shrank by 3%—note that ED did not change at all (Table 1).

The incirrate specimen was a female of *Muusoctopus* sp. collected in the Alpha Fracture Zone of the Bering Sea at 2367–2713 m depth (Werner et al. 2016; Sagorny 2017). This deep-sea finless octopod specimen (ZMB MOLL 240161) with 48 mm ML was photographed fresh on 27 July 2016 (Fig. 6B, left) and subsequently treated in the exact same way as the previous specimen. Directly following capture, the partially semi-transparent body was uniformly white, except for the light-red ventral surface of arms and primary web (Fig. 6B, left). The mantle was ovoid and the overall appearance muscular and robust. All following photographic sessions were executed on the exact same days as for the cirrate specimen mentioned above. On 19 April 2017 (Fig. 6B, center left), shrinkage was most pronounced for ML (2%), while TL shrank by 1% (Table 1). On 22 September 2017 (Fig. 6B, center), shrinkage compared to the fresh specimen was 4% for ML and 3% for HW, while TL was still reduced by 1%. Following transfer to 70% ethanol, shrinkage on 29 November 2017 (Fig. 6B, center right) was still the same as for the previous session, except for TL (2%). On the final photography session on 24 April 2019 (Fig. 6B, right) shrinkage was, compared to the fresh specimen, most pronounced for ML (6%) with the remainder of the morphological parameters unchanged, incl. ED (Table 1).

Discussion

Although common knowledge for anyone who ever observed or analyzed cirrate specimens in museum collections, the imagery shown here provides graphic evidence for the degree of alteration that such delicate material can undergo when being subjected to chemical fixation and long-term preservation. However, fixation and preservation artifacts—in particular shrinkage—are known to occur not only in cirrate, but also in deep-sea incirrate octopods (Voight 2001) as well as cephalopods in general (Cohen 1976; Andriquetto and Haimovici 1988; Voight 1991; O’Shea 1997; González et al. 1998), in addition to other metazoan taxa such as annelids (Howmiller 1972), bivalves (Mills et al. 1981), or tunicates (Nishikawa and Terazaki 1996; Mitchell et al. 2021).

Due to their often semi-gelatinous to gelatinous body composition, cirrates are particularly prone to exhibit the negative side effects of common fixation and preservation methods (Collins and Villanueva 2006). For instance, Collins and Henriques (2000) described how the appearance of specimens of *Stauroteuthis gilchristi*—a cirrate species with particularly gelatinous tissues—changed during preservation, in particular when treated directly with alcohol: in

this scenario, the authors noted general shrinkage of arms and tissues, a significant reduction in TL and PA, suckers appearing much closer together than in specimens analyzed fresh or treated with formalin, tissues being no longer transparent with eyes and internal organs no longer visible, and fins seemingly being located much closer to the apex of the mantle. Unfortunately, such drastic changes to a specimen’s aspect can become scientifically, instead of merely aesthetically problematic, as cephalopod species description and identification rely on precise measurements and calculation of morphometric indices (Roper and Voss 1983). Cirrate taxonomists have become increasingly aware of this issue and have previously sought to quantify the changes occurring during fixation by comparing measurements carried out pre- and post-fixation. Such comparisons provided values for shrinkage of 17–32% ML (Collins et al. 2001) and 30% ML (Guerra et al. 1998) for *Cirrothauma magna* or 28–39% ML and 6–23% TL for *S. gilchristi* (Collins and Henriques 2000) in cirroteuthids. In grimpoteuthids, *Luteuthis shuishi* exhibited a shrinkage of 53% ML (O’Shea and Lu 2002), while *Grimpoteuthis imperator* showed a reduction of 16% ML (Ziegler and Sagorny 2021) and *Cryptoteuthis brevisbracchiata* of 13% ML (Collins 2004) following chemical treatment. The present study expands these findings to selected species from all eight extant cirrate genera and underlines the significant changes that occur in members of this taxon even within relative short periods of time and even if restricted to fixation using buffered formalin. The present morphometric data (Table 1) also support the previous finding that shrinkage does not occur uniformly in all taxonomically relevant structures, thus rendering the use of morphometric indices based on ML alone problematic, at least for museum material. The comparative experiment using cirrate and incirrate adults illustrates that it is indeed the (semi-) gelatinous tissues found in most cirrates that undergo pronounced changes—and significantly more so than the muscular tissues even of deep-sea incirrates (Voight 2001).

Fortunately, the internal anatomy seems to be less affected by shrinkage due to the histological properties of the viscera (Meyer 1906; Ebersbach 1915; Robson 1932; González et al. 1998). For example, Meyer (1906) noted that despite fixation in formalin and preservation in ethanol resulting in a suboptimal state of the internal organs in an adult specimen of *Opisthoteuthis depressa* Ijima & Ikeda, 1895, he was still able to obtain thin sections suitable for histological study. Consequently, internal organs as well as external structures not significantly affected by chemical treatment due to their composition (e.g. suckers, nodules) should be incorporated more consistently into future cirrate species descriptions or re-descriptions of existing taxa, as recently successfully shown for several new species (Verhoeff 2022; Verhoeff and O’Shea 2022). Apart from invasive dissections and time-consuming histological analyses,

results on cephalopod internal anatomy can also be obtained using non-invasive imaging techniques such as magnetic resonance imaging or contrast-enhanced micro-computed tomography (Ziegler et al. 2011, 2018, 2021; Xavier et al. 2015; Shea et al. 2018; Ziegler and Sagorny 2021; Ziegler and Ziegler 2022).

Usually of less taxonomic or systematic, but certainly of ecological and behavioral value are descriptions of a specimen's coloration. Unfortunately, chemical treatment of cirrates results in sometimes significant changes to their original coloration. For example, Aldred et al. (1983) noted that an adult specimen of *Cirrothauma murrayi* Chun, 1911 was "a red brown colour virtually all over the body and arms" when caught alive, while chemical treatment resulted in a purplish color of the preserved specimen. Furthermore, Collins and Henriques (2000) described that in preserved *Stauroteuthis* coloration of the animals was more opaque than in the fresh specimens. In addition, Golikov et al. (2020) noted that in *Opisthoteuthis borealis* Collins, 2005 a live specimen was of a "brick-orange color", while the fixed specimen was reddish and then "brown with a dark-violet color of the oral surface of the web". In fact, Meyer (1906) found that in his preserved specimen of *O. depressa*, the color was not produced by pigment cells, but by a subepithelial layer of connective tissue fibers that stored a dark-brown pigment—he concluded that the color of a living specimen cannot be inferred by observing a preserved specimen due to the structural (and chemical) changes caused by fixative and preservative. The often quite pronounced changes in specimen coloration documented here provide further evidence for the negative effects that both formalin and ethanol can have on the natural coloration of cirrates, suggesting that considerable caution should be used when employing this specific character set for scientific inferences.

Based on the protocols listed here (Table 1) as well as those previously published (Roper and Sweeney 1983, Vecchione et al. 2002), the following recommendations for fixation and preservation of cirrate specimens can be made. Although finned octopods usually arrive dead on deck due to their deep-sea provenance, some species may be caught alive. While no specific protocol on management of pain, suffering, and distress has been published for this specific cephalopod taxon, guidelines applicable to other cephalopods and, more specifically, incirrate octopods should be followed. These include the transfer of the specimen to a container filled with seawater and ethanol or magnesium chloride for anaesthesia and terminal anaesthesia (Messenger et al. 1985; Boyle 2010; Cooper 2011; Andrews et al. 2013; Gleadall 2013; Butler-Struben et al. 2018). In addition to abiding by ethical considerations and laws, this approach avoids rapid specimen contraction, thus enhancing the chances of obtaining good quality material for long-term preservation (Mandy Reid, personal communication).

In addition, Vecchione et al. (1998) describe relaxing and then killing *Cirroctopus* by placing them in iced freshwater on-board ship.

Although shrinkage may still occur even when using low-percentage solutions of formalin buffered in seawater, the present data and previous publications indicate that formalin remains the fixative of choice, at least in the foreseeable future. However, this so-called "formalin paradigm" is being increasingly challenged—mostly due to the carcinogenicity (to the human) of the chemical itself and difficulties with subsequent DNA extraction, but also due to its negative side effects on specimen structure and coloration (Simmons 2019); consequently, new formulas that aim to replace formalin are available on the market (Cannavacciuolo et al. 2021). However, until such novel fixatives have been successfully tested on cirrates or other cephalopods, weak concentrations of formalin (4–5%) buffered in seawater or alternatively Steedman's solution (10% propylene glycol, 2.5% formalin or 1% concentrated formaldehyde, and 0.5–1% propylene phenoxetol in seawater) should be used for fixation (Steedman 1976; Collins and Henriques 2000; Simmons 2014), and preferably also for long-term preservation (Martin A. Collins, personal communication). For fixation, the specimen should ideally be relaxed (see above) and then placed in a container filled with a liquid volume at least five times (Jereb et al. 2014), but preferentially ten times the volume of the specimen—make sure that the specimen is completely covered by the fixative, that its arms are outstretched and that it is neither bent nor folded (Mandy Reid, personal communication). In addition, check whether the fixative has entered the mantle cavity, either by injecting the fixative or by stroking the mantle to release air trapped inside the mantle cavity. If transfer to a long-term preservative on alcohol basis is unavoidable, the specimen should be kept in the formalin fixative for at least two weeks, then rinsed in tap water 2–3 times with vigorous soaking, and finally be transferred to 70% ethanol (or alternatively 40% isopropyl alcohol) using a gradual series.

As the present study illustrates, cirrate specimens in museum collections have been treated using a broad array of methods and protocols over different time scales, making a morphometric and color-based comparison between individuals even of a given species difficult. Nonetheless, certain patterns of shrinkage or loss of color in particular characters (e.g. primary web, arms) could emerge if more material is observed in the future—such data could potentially lead to the establishment of correction factors as recently introduced for specimen weight (Golikov et al. 2020, 2022). Furthermore, while previous as well as the above-mentioned suggestions regarding anaesthetization, fixation and preservation of cirrates are certainly good to know, the reality on-board a research vessel often precludes the exact application of such guidelines. We, therefore, hope that the overview of the effects of fixation

and preservation on finned octopods given here will provide additional stimulus and direction to develop a standardized set of morphological characters that can be used for species description and identification independent of the sometimes drastic artifacts known to be caused by the chemical treatment of finned octopods.

Acknowledgements We would like to thank Jon Ablett, Andreas Allspach, Cheryl A. Brantley, Wen-Sung Chung, Martin A. Collins, Dieter Fiege, Julian K. Finn, Karen Gowlett-Holmes, Bernhard Hausdorf, Ping-Ho Ho, Eric A. Lazo-Wasem, Chung-Chen Lu, Sadie Mills, Claude Nozères, Tim O'Hara, Diana Párrage, Uwe Piatkowski, Andrea Paz Martinez Salinas, Timothy M. Shank, Elizabeth K. Shea, Darren Stevens, Michael Türkay, Michael Vecchione, Tristan J. Verhoeff, Thomas von Rintelen, Regina Wetzler, Catalina Amanda Merino Yunnissi, Christine Zorn, and Robert Zugaro for facilitating access to photographs of fresh and museum specimens. Anna Kral is thanked for discussion of previous versions of this manuscript, while comments by two anonymous reviewers additionally helped to improve the text. We are particularly grateful to the captain, chief scientist, and crew of R/V SONNE during scientific cruise SO-249 BERING for their assistance in obtaining cephalopod specimens.

Author contributions AZ conceived the study. AZ and CS performed measurements. AZ and CS analyzed the data. AZ wrote the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. The present study did not receive any specific funding.

Data/code availability Morphometric data are available in Table 1. All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

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