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**DIFFERENTIAL DISTRIBUTION OF MINERALS  
IN THE PROBOSCIS HOOKS OF *CORYNOSOMA PARAEVAE*  
(ACANTHOCEPHALA, POLYMORPHIDAE)  
JUVENILES FROM *NOTOTHENIA CORIICEPS*  
OFF ARGENTINE ISLANDS, WEST ANTARCTICA**

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**Differential Distribution of Minerals in the Proboscis Hooks of *Corynosoma paraevae* (Acanthocephala, Polymorphidae) Juveniles from *Notothenia coriiceps* off Argentine Islands, West Antarctica.** Amin, O. M., Caracciolo, M. E., Wendt, C., Rubtsova, N. Y., de Souza, W. — Excysted cystacanths of *Corynosoma paraevae* Amin, et al., 2025 (Polymorphidae) were described as a cryptic species from the body cavity of *Notothenia coriiceps* Richardson, 1844 collected off Galindez Island, Argentine Islands. This description included its molecular analysis, SEM images, and Energy Dispersive X-ray Analysis (EDXA) for the first time which demonstrated highest levels of calcium in all hooks and hook roots compared to sulphur and phosphorus. We report here for the first time a new aspect of elemental analysis of hooks demonstrating the differential distribution of sulphur, phosphorous, calcium, and magnesium at the center and margins of anterior, middle and posterior hooks, and hook roots. In this study, Calcium was found to be the most prevalent element in roots (root edge and root middle) and the center of middle hooks. Phosphorous was also common but not as prevalent as calcium and was highest in the center of the roots and also the middle of all hooks. Interestingly, phosphorous was consistent in all hook roots but was lowest in the center of posterior hooks and roots. Sulphur was negligible or absent in root center

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of anterior and middle hooks, pronounced in posterior hook root and most prevalent in the edges of all hook tips as well as the edge of the middle hook. The biological importance of this pattern is discussed in terms relevance to hook strength and flexibility, species identity and comparative systematics. This EDXA profile of *C. paraevae* was distinctly differentiated from that of the morphologically closely related species *Corynosoma pseudohamanni* Zdzitowiecki, 1984.

**Key words:** *Corynosoma paraevae* West Antarctica, EDXA, localization of minerals in hooks, taxonomic implications.

## Introduction

Excysted cystacanths of *Corynosoma paraevae* Amin, Chaudhary, Caracciolo, Rubtsova, Wendt, Kuzmina, de Souza & Singh, 2025 (Polymorphidae) were recently described as a cryptic species from the body cavity of *Notothernia coriiceps* Richardson, 1844 collected off Galindez Island, Argentine Islands (Amin et al., 2025 c). The results of the EDXA of hooks of the closely related *Corynosoma pseudohamanni* Zdzitowiecki, 1984 (Polymorphidae) show the predominance of calcium (11.5–47.0%) and to some extent phosphorus (2.0–19.6%) in all analyzed hooks followed by lower levels of sulphur (0.0–7.1%) (Amin et al., 2025 a). Calcium levels reached the highest levels in the roots of the anterior (50.6%), middle (67.9%), and posterior (51.9%) hooks. Sulphur reached higher levels at the edges of all hooks; however, we only detected the highest concentration of this element at the tip of the middle hook (7.1%). Magnesium exhibited the lowest levels or was mostly undetectable (0.0–1.2%). Notably, in the anterior hook, magnesium was only detected in the root (1.5%). In the present study of *C. paraevae*, the relative prevalence of calcium, phosphorous, sulphur and magnesium were somewhat comparable to those of *C. pseudohamanni* but distinctly different in the center and the edge of each of the anterior, middle and posterior hooks and hook roots.

Cystacanths and juveniles of five other species of acanthocephalans were studied by EDXA for elemental composition. These are (1) *Southwellina hispida* (Van Cleave, 1925) Witenberg, 1932 (Polymorphidae) from the long jaw mudsucker *Gillichthys mirabilis* Cooper (Gobiidae) off the California coast (Amin et al., 2022 a, b); (2) cystacanths of *Profilicollis rancoensis* Amin, Rodríguez, Farrer, Fierro, Garcés, Rivera, D'Elía, 2023 (Polymorphidae) from the freshwater crab *Aegla abtao* Schmitt (Crustacea: Decapoda) in Ranchi Lake, North Patagonia, Chile (Amin et al., 2023 b); (3) cystacanths of *Neoandracantha peruensis* Amin, Heckmann, 2017 from the ghost crab *Ocypode quadrichaudi* Milne-Edwards and Lucas in Peru (Amin and Heckmann, 2017); (4) cystacanths of *Sphaerirostris picae* (Rudolphi, 1819) Golvan, 1956 from lizards and hedgehogs in Ukraine (Amin et al., 2023 a); and (5) Cystacanths of *Moniliformis kalahariensis* Meyer, 1931 from *Blatella germanica* Linn. in India (Amin et al., 2023 a). We describe herein, the mineral distribution in the margins and center of the anterior, middle and posterior proboscis hooks and hook roots of cystacanths of *C. paraevae* for the first time.

## Material and Methods

### Collections

A total of 34 cystacanths mostly of *C. paraevae* and some individuals of *C. evae* were collected from cysts in the body cavity of 5 individuals of the Atlantic black rockcod *No-*

*tothenia coriiceps* between November 2022 and March 2023 off Galindez Island, Argentine Islands, West Antarctica (65°15' S, 64°15' W). This collection was part of the long-term parasitological monitoring studies carried out since 2014–2015 by the Ukrainian Antarctic expeditions at the Ukrainian Antarctic station (UAS) “Akademik Vernadsky” on Galindez Island, West Antarctica. Acanthocephalan cysts were collected manually from fish body cavity sites, washed in saline after excystation, and then kept for a few hours in saline for proboscis evagination before fixing in 70% ethanol. We have processed 10 specimens of each sex for SEM and Energy Dispersive x-ray analysis (EDXA).

### Deposits

Voucher specimens were deposited in Harold W. Manter Laboratory (HWML) collection of the University of Nebraska's State Museum's in Lincoln, Nebraska, USA; collection no. HWML 217883. Additional specimens are kept in 70% ethanol in the OMA collection.

### Energy Dispersive X-ray analysis (EDXA)

EDXA is an analytical technique associated with electron microscopy, used for the elemental analysis or chemical characterization of a sample. It relies on the interaction between an X-ray excitation source and the sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic emission spectrum [https://en.wikipedia.org/wiki/Energy-dispersive\\_X-ray\\_spectroscopy-cite\\_note-Goldstein-2](https://en.wikipedia.org/wiki/Energy-dispersive_X-ray_spectroscopy-cite_note-Goldstein-2) (Goldstein, 2003; Scimeca et al., 2018) which is the main principle of spectroscopy. A similar methodology to study the hooks *Moniliformis kalahariensis* Meyer, 1931 (Moniliformidae Van Cleave, 1924) was combined with TEAM (Texture and Elemental Analytical Microscopy) which is a technique that combines both crystallographic texture analysis (using Electron Backscatter Diffraction — EBSD) and elemental analysis (using Energy Dispersive X-ray Spectroscopy — EDS) on a single electron microscope platform (Tan, 2024). This technique allowed us to simultaneously study the microstructure and chemical composition of the *M. kalahariensis* material at the micro-scale (Amin et al., 2025 b).

The fixed samples were dehydrated through an ascending ethanol series and then critical point dried using a Tousimis Autosamdri-815 (Tousimis Research Corporation, Rockville, United States). They were mounted on stubs and coated with carbon using a sputter coater Balzers MED 010 (Balzers Union limited, Balzers, Liechtenstein.) establishing an approximate thickness of 20 nm. The specimens were examined and positioned using the LYRA3 FIB-SEM (TESCAN, Brno-Kohoutovice, Czech Republic), equipped with a Phoenix energy-dispersive X-ray analyzer (Oxford Instruments, Abingdon, England). X-ray spot analysis and live scan analysis were performed at 15 kV with a spot size of 2, and the results were recorded on charts and stored using digital imaging software attached to a computer. The AZtec version 4.3 software system (Oxford Instruments, Abingdon, England) was used.

A dual-beam SEM with a gallium (Ga) ion source was used for the LIMS (Liquid Ion Metal Source) part of the process. The hooks of the acanthocephalans were centered on the SEM stage and sectioned longitudinally. The cut was then analyzed with X-rays at the tip, middle, and base of the hooks for chemical ions using an electron beam (Tungsten) to obtain an X-ray spectrum.

SEM data maps were created marking spectra from the edge and middle of hook tips, middle and roots followed by color-coded spectra for magnesium, calcium, phosphorous and sulphur showing element overlay then graphs and tables for each spectrum of anterior hooks (Figs 1–11, here and further pictures see in the Appendix), middle hooks (Figs 12–23) and posterior hooks (Figs 24–34).

## Results

EDXA analysis of elements in anterior, middle and posterior hooks is depicted in figures 1–12, 13–24 and 25–36, respectively. Our results are reported as weight % for calcium, phosphorous, sulphur and magnesium. Atomic % and K ratios are also reported in all tables. Common elements in living cells (H, O, N) and cutting elements (Ga) are also listed in all tables and spectra but not discussed. Element prevalences are given for anterior hooks (Figs 6–12), middle hooks (Figs 18–24), and posterior hooks (Figs 30–34) hooks. Calcium was the most prevalent element in the center of all roots reaching 50.2%, 48.62% and 20.45% in anterior, middle and posterior hook roots and also in the edge of anterior hook root reaching 42.18% but considerably lower in the middle and posterior roots reaching only 14.13% and 8.51%, respectively. Calcium levels were considerably higher in the center of anterior, middle and posterior hooks being 47.31 %, 34.47% and 18.71%, respectively compared to at the edge of the same hooks, 20.88%, 17.03 and lowest at 1.82 % in the same order. The tip hook center also had highest calcium levels compared to tip hook edge being 32.27% vs. 11.42% in anterior hooks, 25.07 vs. 4.62% in middle hooks, and 7.45% vs. 0.73% in posterior hook.

Phosphorous was equally common but not as prevalent as calcium and was higher in the center of the middle and posterior hook roots compared to the edge of these roots: 21.86% vs. 8.01% and 8.81 vs. 4.73%, respectively. Its prevalence was, however, almost equal at the root center (21.69%) and the root edge (22.96%). It was also more prevalent in middle hook center compared to middle hook edge being 24.1% vs. 13.37, 19.33% vs. 11.72%, and 12.06% vs. 1.83% in anterior, middle, and posterior hooks, respectively. The same pattern was repeated in hook tips: 21.30% vs. 9.24%, 15.17% vs. 2.19% and 4.59% vs. 0.53% in the same order. Interestingly, phosphorous was present in all hooks and hook roots but was progressively lower in more posterior locations.

Sulphur was negligible in anterior and middle root center but prominent in middle hook root edge at 9.18% and equally prominent in posterior hook root center (14.07%) and edge (14.27%). It was highest at the tip edge of middle hook reaching 18.74% and somewhat lower in the same location in the anterior hook (9.29%) and posterior hook (6.69%).

## Discussion

### Energy Dispersive X-ray Analysis (EDXA)

Our studies of acanthocephalan worms have usually involved X-ray scans (EDXA) of FIB-sectioned hooks and spines. The chemical elements present in the hooks are typical for acanthocephalans. Hooks were evaluated for chemical ions, with sulphur (s), calcium (ca) and phosphorus (p) being the prominent elements. Calcium and phosphorus are major ions at the base and middle of the hooks that play a prominent

role in host tissue attachment (Heckmann, et al., 2007, 2012; Standing and Heckmann, 2014; Amin and Heckmann, 2017). Sulphur is usually high especially at the outer edge of large hooks and hook tips. These elements are critical for the mineralization of the hook which creates the hardened outer layer, an apatite, similar to tooth enamel of mammals. Magnesium probably plays a role in the mineralization of hooks similar to the disulfide bonds formed by sulphur in the protein apatite (Amin and Heckmann, 2017). Sodium, a rarely prominent metal, was found in scans of whole hooks of some other acanthocephalans.

Comparative findings

Our results support the above findings and are related to those of the closely related *Corynosoma pseudohamanni* also collected from *N. coriiceps* in Galindez Island, West Antractica. The morphological and molecular distinctions between cystacanths of these two species, *C. paraevae* and *C. pseudohamanni* are further supported by the unique EDXA profile of each species comparatively summarized in Table 1. Distinctions are also noted among cystacanths of 5 other species highlighted in the Introduction.

Biological significance

EDXA, as a diagnostic tool, supports the observation that populations of an acanthocephalan species will consistently have similar EDXA spectra irrespective of host species or geography. The taxonomic identity of species is deep-seated at the genetic level manifesting the organism’s morphology and biochemistry as revealed, in part, by its elemental spectra (Amin et al., 2022 a, b).

The results of large and small gallium (Ga) cut anterior hooks (Figs 6–12), middle hooks (Figs 18–24), and posterior hooks and roots (Figs 30–34) are given. The elements necessary for the mineralization and hardening of the hooks especially calcium and phosphorus are present with sulphur in minimum amounts. In contrast,

Table 1. Comparative assessment of marked differences in EDXA patterns in proboscis hooks of *Corynosoma pseudohamanni* and *Corynosoma paraeva*

Element by Wt, %	<i>Corynosoma pseudohamanni</i> , %	<i>Corynosoma paraevae</i> , %	Hook position and phase cut
Sulphur	1.94	9.29	Anterior hook tip edge
	7.06	18.74	Middle hook tip edge
	0.0	9.18	Middle hook root edge
	1.46 & 1.11	14.27 & 14.07	Post. hook root edge & root center
Calcium	14.05	4.62	Middle hook tip edge
	61.83	14.13	Middle hook root edge
	38.17	18.71	Post. hook middle center
	16.92	1.82	Post. hook middle edge
	48.55	8.51	Post hook root edge
Phosphorous	2.03	13.37	Middle hook center edge
	11.43	24.10	Middle hook middle center
	9.34	1.83	Post. hook middle edge
Magnesium	0.0	1.93	Anterior hook middle edge
	0.0	2.04	Middle hook middle center
	0.85	1.62	Post. hook middle center



the percent of sulphur was highest in hook tips and hook edges of *Cavisoma magnum* (Southwell, 1927) Van Cleave, 1931 from *Mugil cephalus* in the Arabian Sea reaching 43.51 wt. % and 27.46 wt. %, respectively (Amin et al., 2018).

Metal analysis of hooks has become a diagnostic standard since hooks have the highest level of elements compared to the mid- and posterior trunk regions of the acanthocephalan body (Heckmann et al., 2012). Specifically, the sulphur content in the proboscis is paramount in the composition of disulfide bonds in the thiol groups for cysteine and cystine of the polymerized protein molecules. Protein synthesis occurs in two stages, transcription and translation (Stegman, 2005). The formed disulfide bonds are direct by-products of the DNA-based process of protein synthesis which makes up the identity of a biological species. Accordingly, the level of sulphur, in our EDXA profiles, will indicate the number of sulphur bonds that along with the levels of calcium phosphates, will characterize the identity of a species based on its nuclear DNA personality (Amin et al., 2022 a, b). Diversity in the species-specific EDXA profiles will further substantiate its taxonomic relevance in both the cystacanth and adult stages, when such information becomes available.

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#### **Authors contributions**

**Conflicts of interest.** None

**Ethical standards.** The authors declare that they have observed all applicable ethical standards.

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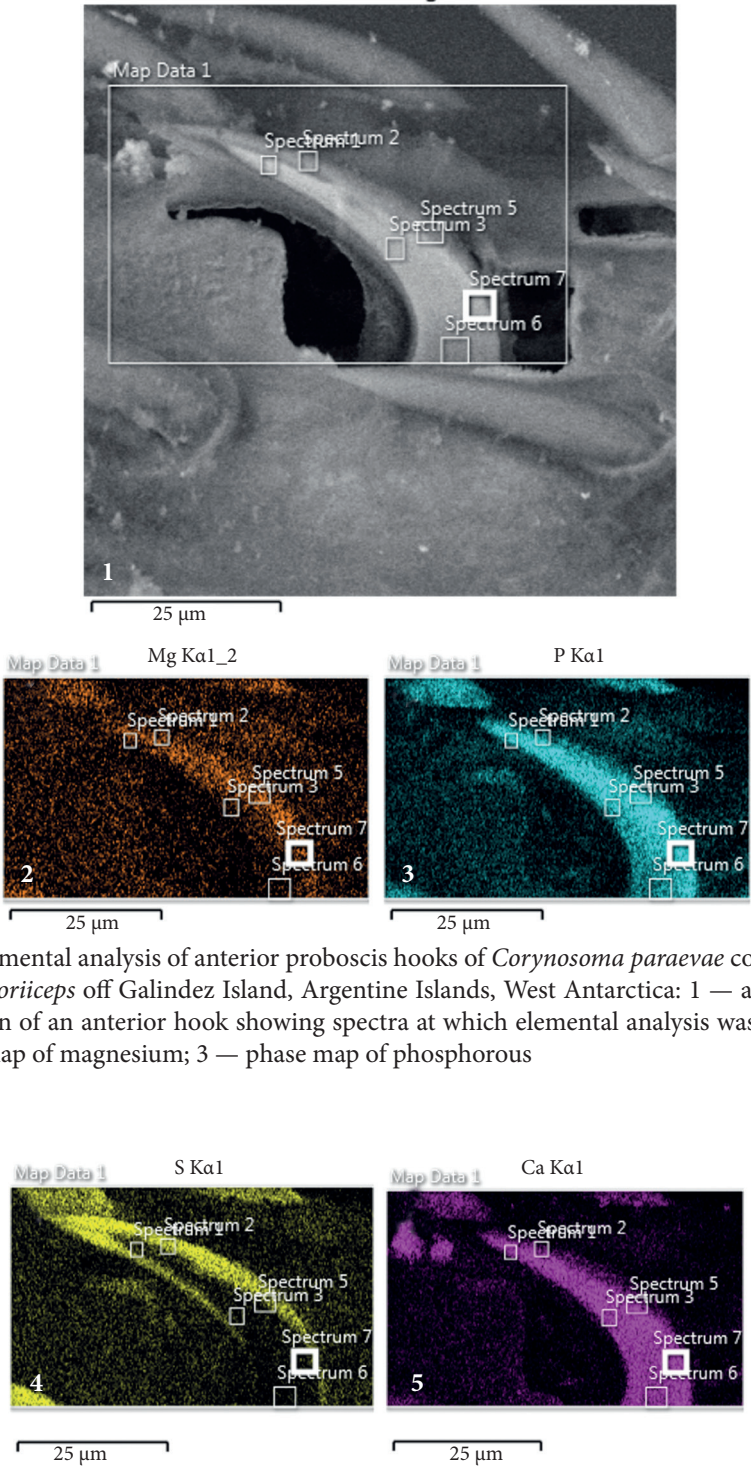
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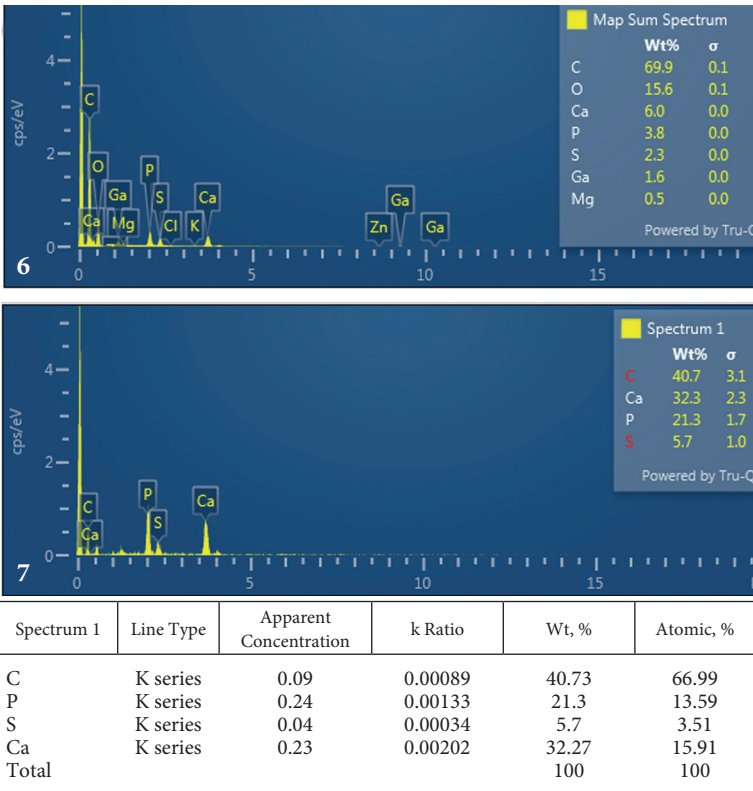
Appendix



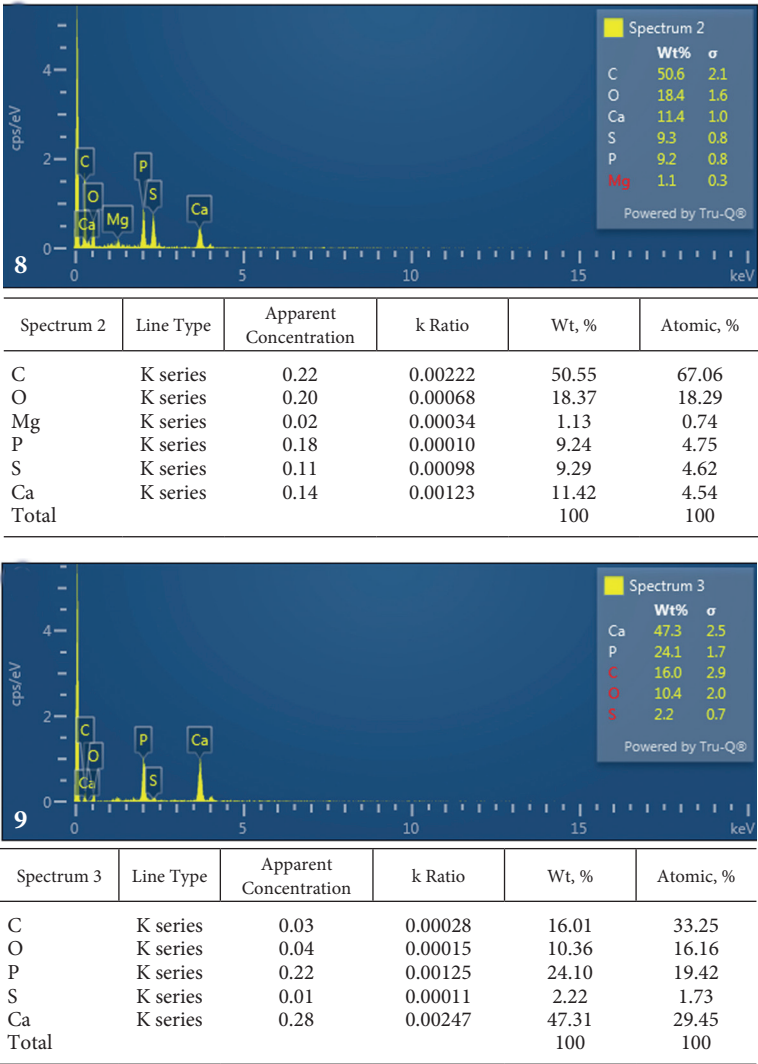
**Figs 1–3.** Elemental analysis of anterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 1 — a gallium-cut lateral section of an anterior hook showing spectra at which elemental analysis was performed; 2 — phase map of magnesium; 3 — phase map of phosphorous

**Figs 4–5.** Elemental analysis of anterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 4 — phase map of sulphur; 5 — phase map of calcium

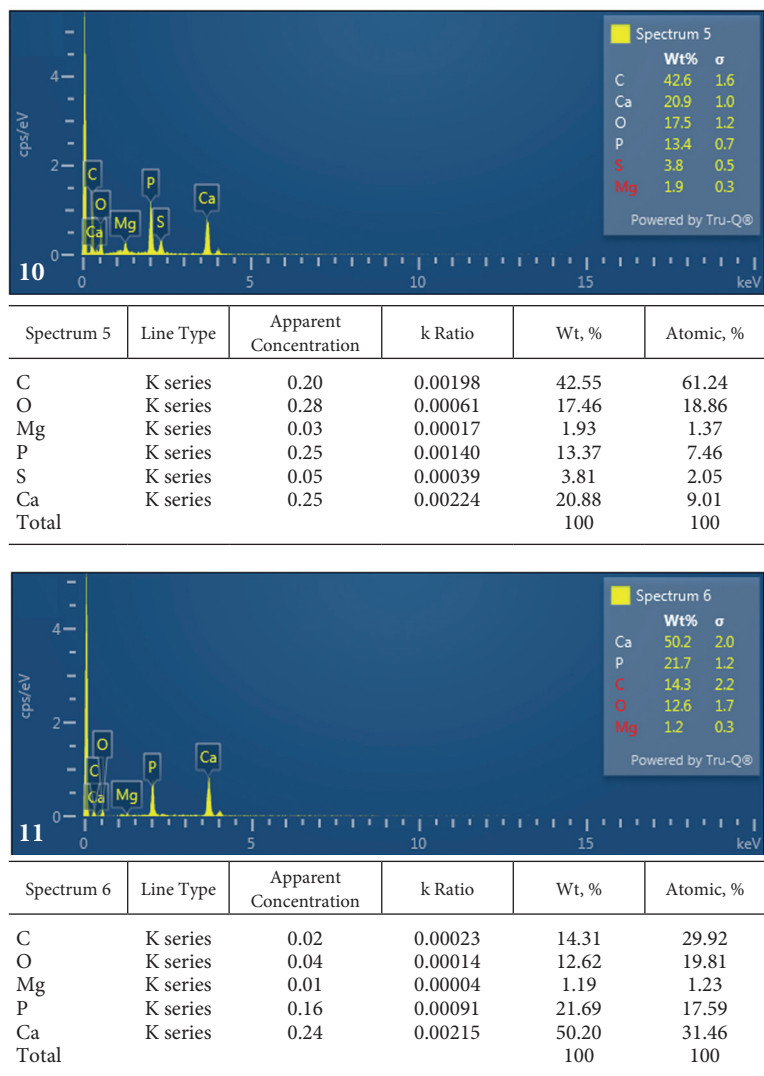




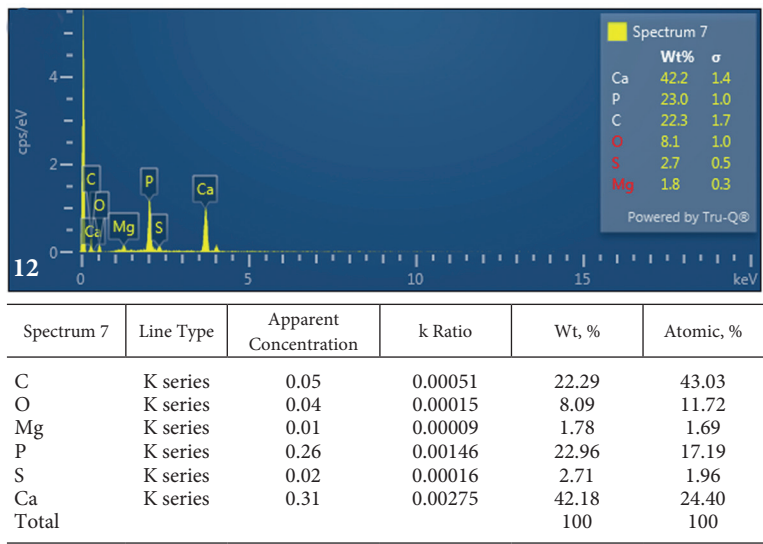
**Figs 6–7.** Elemental analysis of anterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 6 — map of sum spectra with the inset showing the numerical analysis of the spectra; 7 — graphic and tabulated levels of elements analyzed from the hook tip center (spectrum 1)



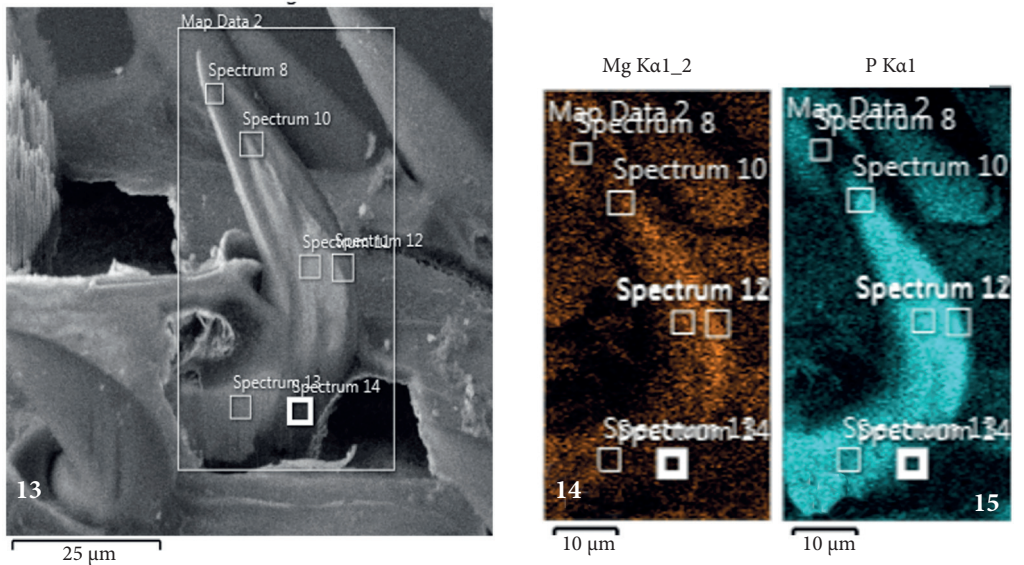
**Figs 8–9.** Elemental analysis of anterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 8 — graphic and tabulated levels of elements analyzed from the hook tip edge (spectrum 2); 9 — graphic and tabulated levels of elements analyzed from the center of the hook middle (spectrum 3)



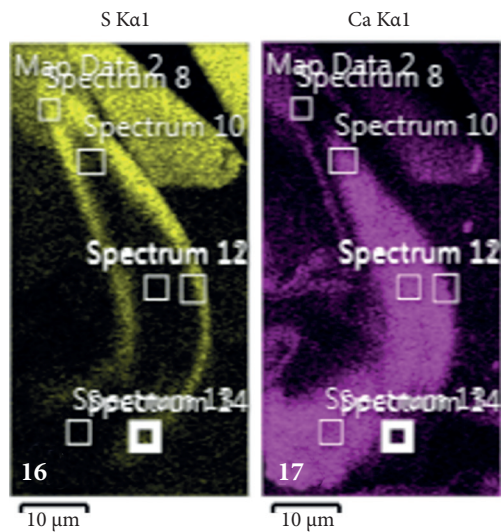
**Figs 10–11.** Elemental analysis of anterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 10 — graphic and tabulated levels of elements analyzed from the edge of the hook middle (spectrum 5); 11 — graphic and tabulated levels of elements analyzed from the hook root center (spectrum 6)



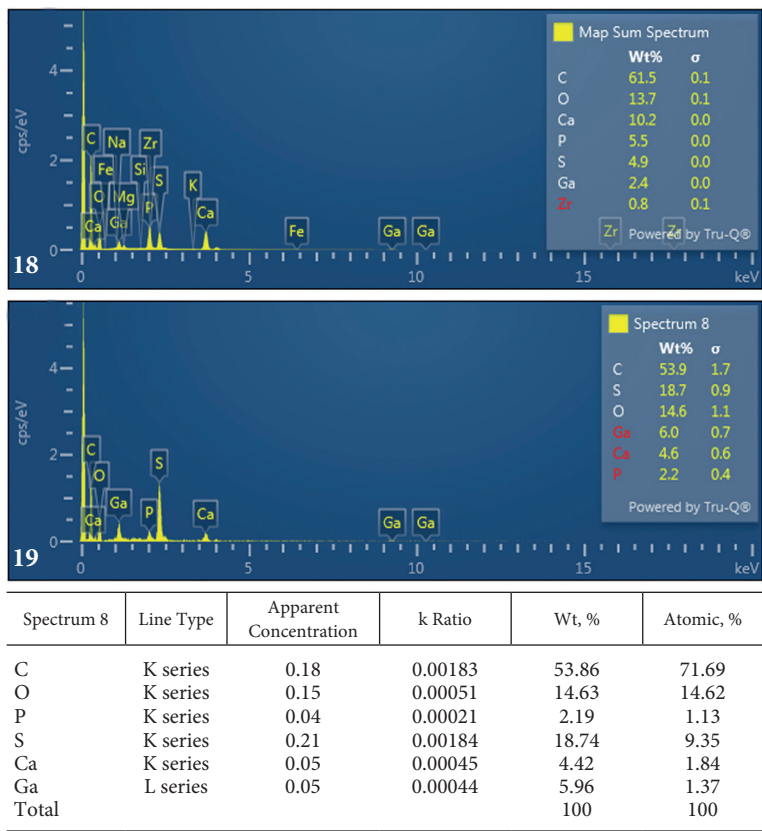
**Fig 12.** Elemental analysis of anterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: graphic and tabulated levels of elements analyzed from the edge of the hook root (spectrum 7)



**Figs 13–15.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 13 — a gallium-cut lateral section of a middle hook showing spectra at which elemental analysis was performed; 14 — phase map of magnesium; 15 — phase map of phosphorous

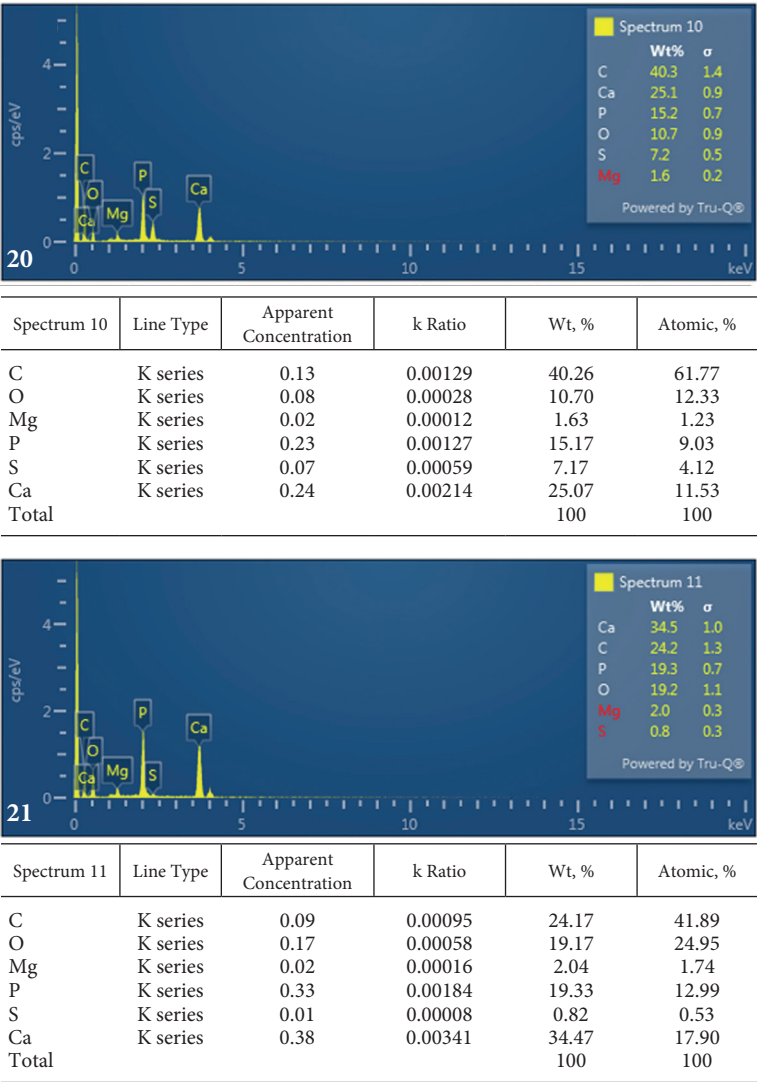


**Figs 16–17.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 16 — phase map of sulphur; 17 — phase map of calcium

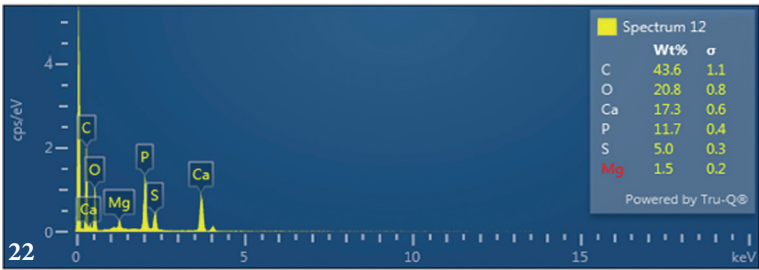


**Figs 18–19.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 18 — map of sum spectra with the inset showing the numerical analysis of the spectra; 19 — graphic and tabulated levels of elements analyzed from edge of hook tip (spectrum 8)

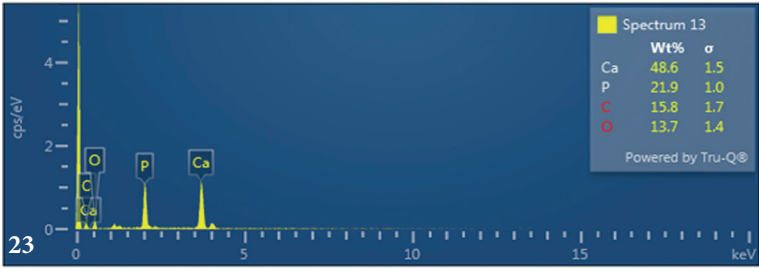




**Figs 20–21.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 20 — graphic and tabulated levels of elements analyzed from the center of hook tip (spectrum 10); 21 — graphic and tabulated levels of elements analyzed from the center of the hook middle (spectrum 11)

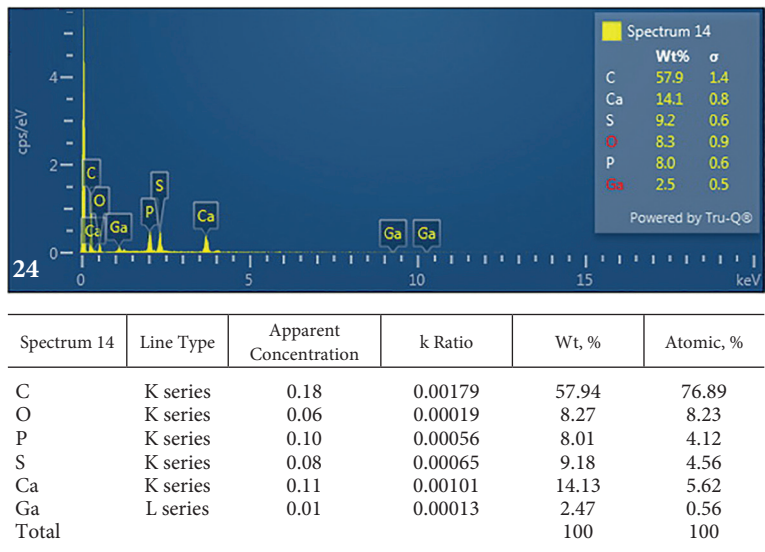


Spectrum 12	Line Type	Apparent Concentration	k Ratio	Wt, %	Atomic, %
C	K series	0.26	0.00260	43.62	60.91
O	K series	0.29	0.00097	20.84	21.85
Mg	K series	0.03	0.00017	1.53	1.05
P	K series	0.28	0.00156	11.72	6.34
S	K series	0.08	0.00066	5.00	2.61
Ca	K series	0.26	0.00236	17.30	7.24
Total				100	100

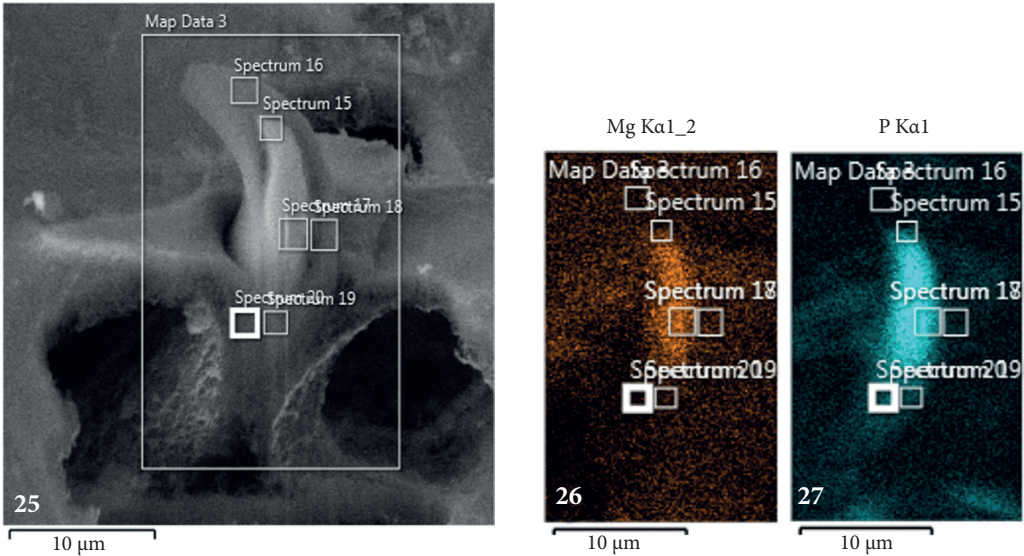


Spectrum 13	Line Type	Apparent Concentration	k Ratio	Wt, %	Atomic, %
C	K series	0.04	0.00036	15.84	32.21
O	K series	0.07	0.00023	13.69	20.90
P	K series	0.23	0.00131	21.86	17.24
Ca	K series	0.33	0.00298	48.62	29.64
Total				100	100

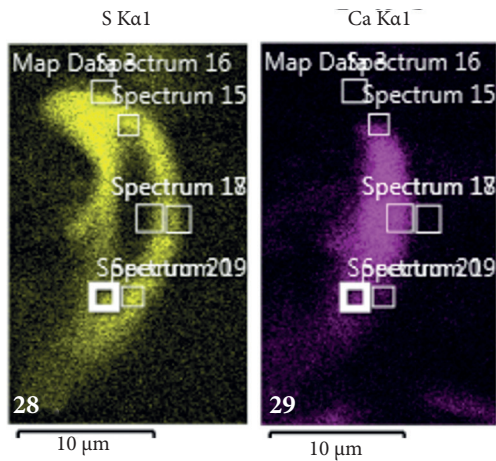
**Figs 22–23.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothernia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 22 — graphic and tabulated levels of elements analyzed from the edge of the hook middle (spectrum 12); 23 — graphic and tabulated levels of elements analyzed from the center of hook root (spectrum 13)



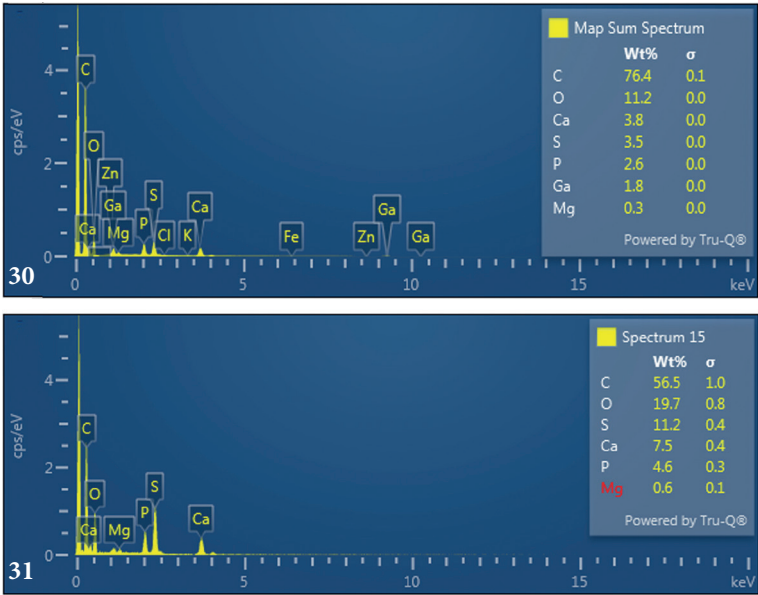
**Fig. 24.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: graphic and tabulated levels of elements analyzed from the edge of the hook root (spectrum 14)



**Figs 25–27.** Elemental analysis of posterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 25 — gallium-cut lateral section of a posterior hook showing spectra at which elemental analysis was performed; 26 — phase map of magnesium; 27 — phase map of phosphorous

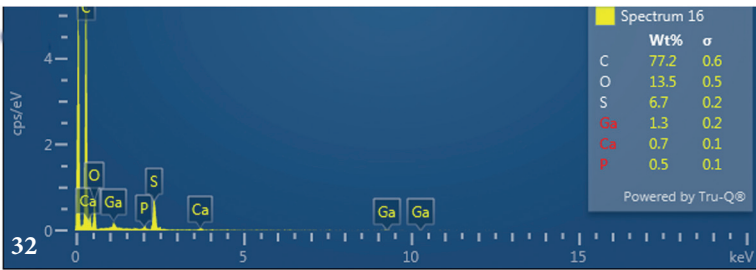


**Figs 28–29.** Elemental analysis of middle proboscis hooks of *Corynosoma paraevae* collected from *Notothernia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 28 — phase map of sulphur; 29 — phase map of calcium

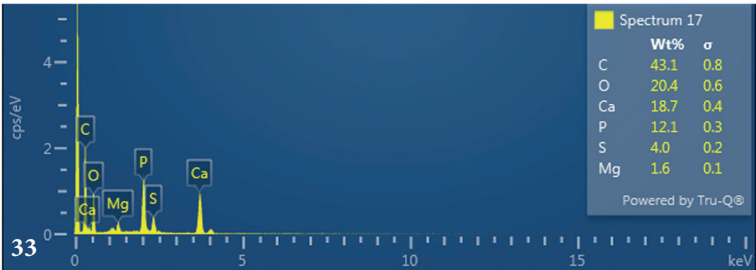


Spectrum 15	Line Type	Apparent Concentration	k Ratio	Wt, %	Atomic, %
C	K series	0.33	0.00331	56.50	70.84
O	K series	0.28	0.00093	19.66	18.50
Mg	K series	0.01	0.00006	0.56	0.35
P	K series	0.11	0.00061	4.59	2.23
S	K series	0.18	0.00152	11.23	5.28
Ca	K series	0.11	0.00100	7.45	2.80
Total				100	100

**Figs 30–31.** Elemental analysis of posterior proboscis hooks of *Corynosoma paraevae* collected from *Notothernia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 30 — map of sum spectra with the inset showing the numerical analysis of the spectra; 31 — graphic and tabulated levels of elements analyzed from the edge of hook tip (spectrum 15)



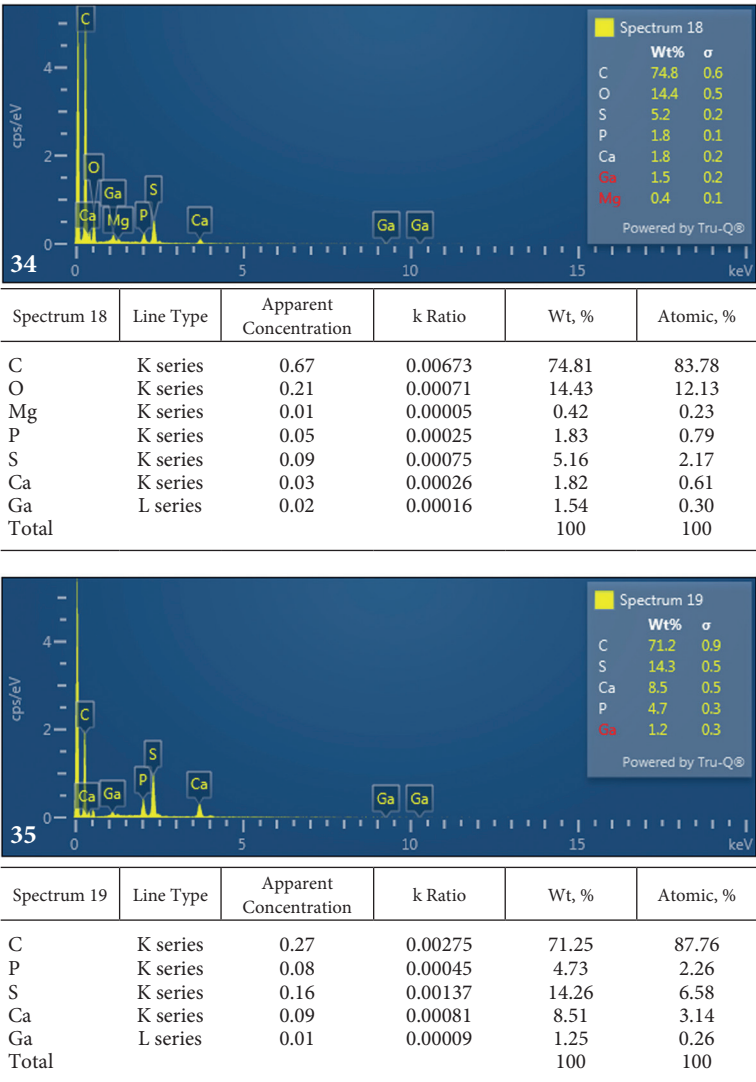
Spectrum 16	Line Type	Apparent Concentration	k Ratio	Wt, %	Atomic, %
C	K series	0.71	0.00707	77.23	85.31
O	K series	0.20	0.00068	13.51	11.21
P	K series	0.01	0.00008	0.53	0.22
S	K series	0.12	0.00100	6.69	2.77
Ca	K series	0.01	0.00011	0.73	0.24
Ga	L series	0.02	0.00014	1.32	0.25
Total				100	100



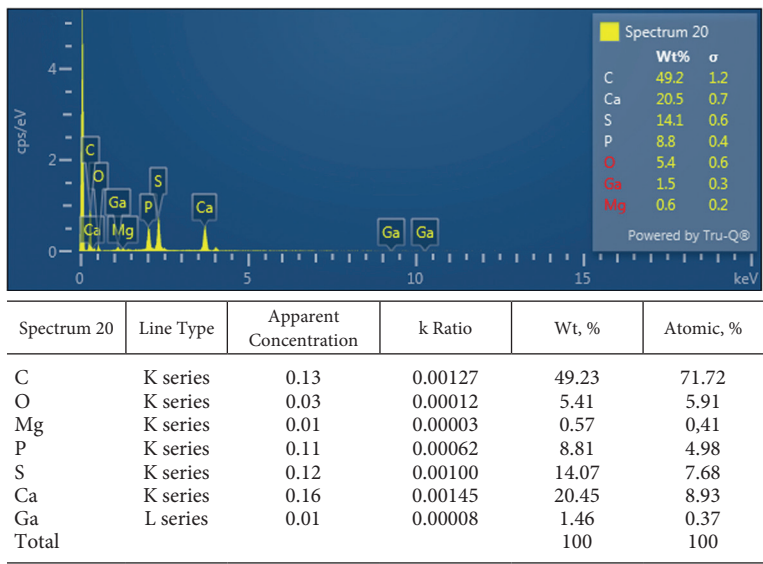
Spectrum 17	Line Type	Apparent Concentration	k Ratio	Wt, %	Atomic, %
C	K series	0.26	0.00259	43.15	60.70
O	K series	0.28	0.00093	20.43	21.58
Mg	K series	0.03	0.00018	1.62	1.13
P	K series	0.28	0.00158	12.06	6.58
S	K series	0.06	0.00052	4.02	2.12
Ca	K series	0.28	0.00252	18.71	7.89
Total				100	100

**Figs 32–33.** Elemental analysis of posterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 32 — graphic and tabulated levels of elements analyzed from hook tip center (spectrum16); 33 — graphic and tabulated levels of elements analyzed from the center of the hook middle (spectrum 17)





**Figs 34–35.** Elemental analysis of posterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica: 34 — graphic and tabulated levels of elements analyzed from the edge of the hook middle (spectrum 18); 35 — graphic and tabulated levels of elements analyzed from the edge of hook root (spectrum 19)



**Fig. 36.** Elemental analysis of posterior proboscis hooks of *Corynosoma paraevae* collected from *Notothenia coriiceps* off Galindez Island, Argentine Islands, West Antarctica. 34. Graphic and tabulated levels of elements analyzed from the hook root center (spectrum 14)