



Description of buoyant fibers adhering to *Argonauta nouryi* (Cephalopoda: Argonautidae) collected from the stomach contents of three top predators in the Mexican South Pacific

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ABSTRACT

Argonauta nouryi Lorois, 1852 is an octopus that inhabits the holopelagic zone, the objective of this study was to describe the occurrence of buoyant fibers adhering to the body and mantle cavity of *A. nouryi* females found in the stomach contents from *Euthynnus lineatus* (skipjack), *Coryphaena hippurus* (dolphinfish), and *Istiophorus platypterus* (sailfish). Stomach contents from 224 individuals were examined. All female evaluated presented fibers adhering to the mantle cavity; 92.6% of the fibers measured 0.25 to 5 mm in length and hyaline was the dominant color (72%). The amount of fibers in the fish stomach contents with *A. nouryi* was significantly greater than in stomachs without; this suggests that the fibers might be introduced via *A. nouryi*. Findings of this work could be related to the discharge of solid materials in the water column.

1. Introduction

The impact on marine ecosystems is related to global ocean pollution, particularly on the ocean surface. Marine organisms, including different species of zooplankton, mollusks, crustaceans, fishes, sea turtles, seabirds, and marine mammals, are threatened mainly through entanglement and the ingestion of plastics (Mathalon and Hill, 2014; Romeo et al., 2015; Alomar and Deudero, 2017; Li et al., 2018). With plastics already present in a diversity of seafood items, there is strong evidence suggesting the transfer of microplastic particles to humans (Carbery et al., 2018). Nonetheless, the occurrence of microplastics in seafood has not yet been quantified or regulated (Ziccardi et al., 2016).

Large plastic items undergo fragmentation processes mainly via UV photo-degradation, wave action, and physical abrasion which result in microplastics (particles < 5 mm) (Song et al., 2017). The most prominent microplastic forms contaminating the marine environment are irregular fragments, pellets, spheres and fibers (filaments) (Mathalon and Hill, 2014).

A major concern related to fragmentation is the disposability of microplastics, these may also be ingested indirectly as a result of trophic transfer when contaminated prey are consumed by predators (Farell and Nelson, 2013; Nelms et al., 2018). Several studies have demonstrated that once ingested, microplastics can accumulate and also move between body tissues via translocation (Browne et al., 2008;

Van Cauwenbergh and Janssen, 2014; Avio et al., 2015), which can result in a variety of adverse effects on the organism (Guzzetti et al., 2018).

Plastic debris collected by surface nets are mostly fragments of bags, containers, packaging, and fishing gear made of polyethylene and polypropylene (e.g., Hidalgo-Ruz et al., 2012; Reisser et al., 2013), and fibers from textile (Mathalon and Hill, 2014). These polymers are less dense than seawater and float on the surface (Reisser et al., 2015). These buoyant fibers can adhere to holopelagic organisms such as Argonauts and keep in together on the sea surface.

The Argonauts are pelagic octopods of the family Argonautidae, only the females of the species construct the paper nautilus shells that serve as brood chambers for developing embryos (Norman, 2000). Argonauts trap air inside their shells on the ocean surface to attain neutral buoyancy; they exhibit extreme sexual dimorphism, males live in the water column and are considerably smaller than females, rarely exceeding 1 cm (Roper et al., 1984).

Females Argonauts are commonly consumed by a number of large epipelagic fish species (e.g. Arizmendi-Rodríguez et al., 2006; Markaida and Sosa-Nishizaki, 2010; Tripp-Valdez et al., 2010; Ortega-García et al., 2017). In the Mexican South Pacific, Noury's argonaut (*Argonauta nouryi*) have been reported in large numbers in the diets of *Coryphaena hippurus* Linnaeus, 1758, *Euthynnus lineatus* Kishinouye, 1920, *Istiophorus platypterus* Shaw, 1792, *Thunnus albacares* Bonnaterre, 1788, and

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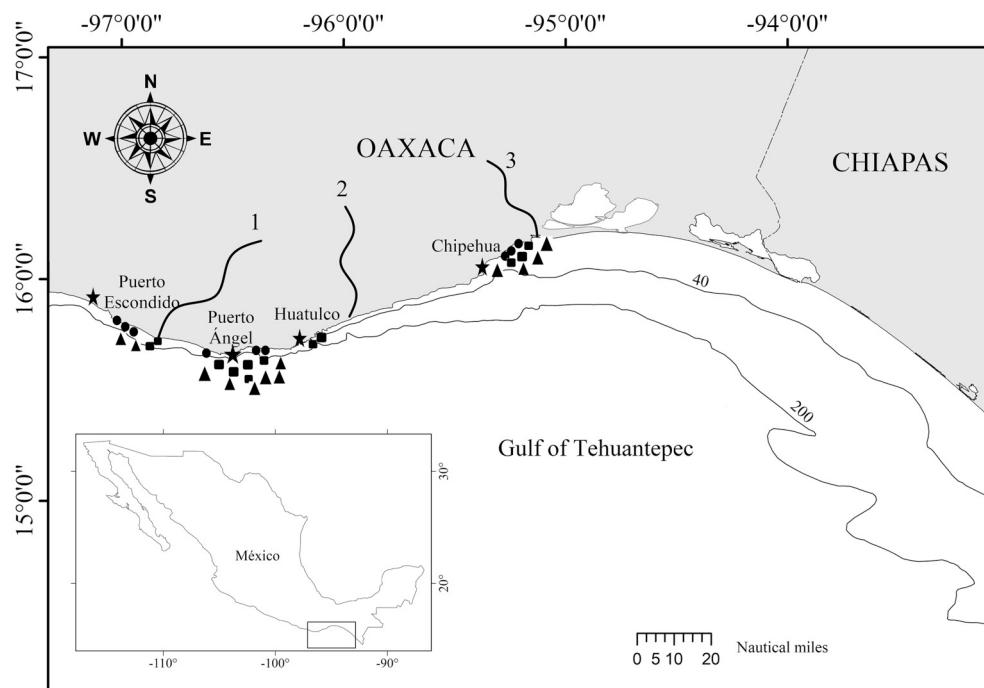


Fig. 1. Map of the Gulf of Tehuantepec, area down to 200 m isobaths. The circles denote fishing grounds for *E. lineatus*; squares and triangles for *I. platypterus* and *C. hippurus*. Rivers: Colotepec (1), Copalita (2), and Tehuantepec (3).

Carcharhinus falciformis Bibron, 1839 (Alejo-Plata et al., 2019). Distributed from southern California to Peru, female Noury's argonaut inhabits the holopelagic zone (Roper et al., 1984).

The goals of this study were to describe the occurrence of buoyant fibers adhering to the body and mantle cavity of *Argonauta nouryi* females found in the stomach contents of three top predators: *E. lineatus*, *C. hippurus*, and *I. platypterus*; identify possible trophic transfer of fibers through the prey *A. nouryi*; and assess the differences between species with ingestion of *A. nouryi* and those without. These large epipelagic fishes are prevalent in Mexican South Pacific catches and are important to human consumption. Moreover, *C. hippurus* and *I. platypterus* are highly migratory species in the Eastern Tropical Pacific.

2. Materials and methods

2.1. Study area

The study area is located in the central western Gulf of Tehuantepec, in the Mexican Tropical Pacific (Fig. 1). It is characterized by a very narrow continental shelf (4 to 6 km) with a steep slope; depths exceeding 4000 m can be reached a short distance from the coastline, providing access to larger pelagic fish present in the area. A tropical site, this area is influenced by warm ocean currents with an average monthly sea surface temperature (SST) above 26 °C throughout the year (Trasviña et al., 1995).

2.2. Sample collection

Stomach contents were collected from *E. lineatus*, *C. hippurus* and *I. platypterus* (Table 1). Samples were obtained monthly from commercial

Table 1

Species, total of sampling, length *I. platypterus* (eye-fork), occurrence (%) of fish with fibers, mean (\pm SD), * only whit whole *A. nouryi*. Number of fibers among species is significantly different (Kruskal-Wallis test, $p < 0.05$).

Large pelagic fish			
	<i>I. platypterus</i>	<i>C. hippurus</i>	<i>E. lineatus</i>
# stomachs	98	32	47
Furcal length (cm)	35–65	20–145	128–225
Stomachs without <i>A. nouryi</i>	12	10	18
Stomachs whit beaks of <i>A. nouryi</i>	54	0	41
Stomachs whit whole <i>A. nouryi</i>	32	22	12
Occurrence (%)	53.8	22.2	24
# fibers	1637	676	730
# fibers per fish *	102 \pm 219.5	62 \pm 78.3	122 \pm 102.8

artisanal catches at different landing sites along the coast of Oaxaca from January 2017 to December 2018. The small-scale fisheries use trolling lines (skipjack); surface longlines and driftnets (sailfish and dolphinfish). All specimens were transported on ice to the docks. Body sizes of fishes were measured (cm); stomach were removed, deposited in aluminum foil bag, and placed on ice and transported to the laboratory.

To minimize contamination in the laboratory, nitrile gloves and cotton lab coats were worn during the work. All work surfaces were wiped down with 70% ethanol prior to stomach dissection. The stereomicroscope area was cleaned and covered with aluminum foil prior to sample analysis. One procedural blank was performed simultaneously during fibers extraction and inspection to assess the

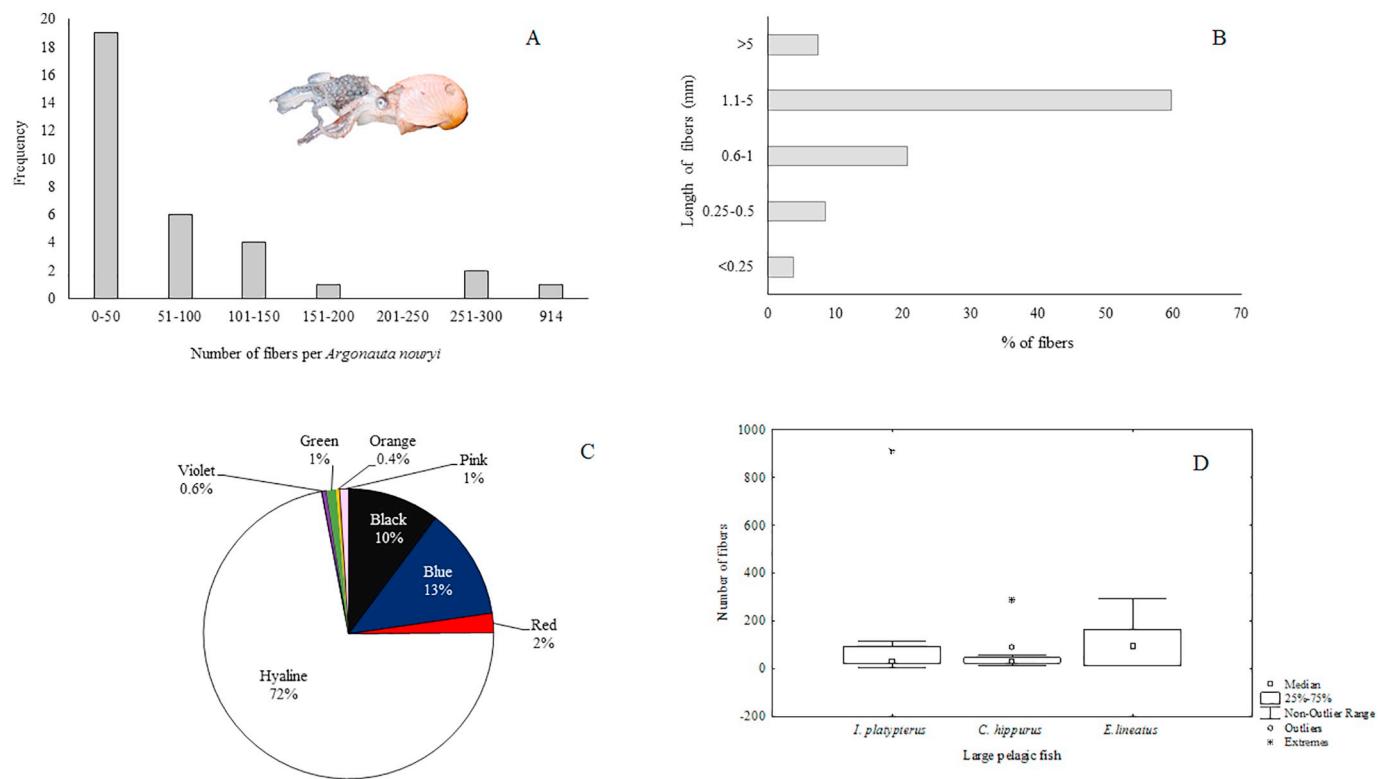


Fig. 2. Fibers found in stomach contents with whole *Argonauta nouryi*. A. Frequency histogram showing number of fibers per Noury's argonaut; B. Percentage (%) contribution of the fibers size classes; C. Pie charts showing the colors of fibers; D. Number of fibers found per fish. Box plots show the median, 25, 75th percentiles in sailfish, dolphinfish and skipjack.

contamination from airborne particles (Alomar et al., 2016). In case of airborne contamination of procedural blanks, the same typology of fibers, according to shape, color and size were removed from results.

All liquids used for analysis were passed through 0.7 mm membrane filters. Vessels and tools were rinsed three times with filtered distilled water and covered with aluminum foil before and after use. Stomachs of the fish were carefully dissected, only whole and undigested *A. nouryi* females were extracted and placed in aluminum foil envelopes and stored at -20°C for subsequent analysis. Fibers were only recovered from within the Noury's argonauts and not the rest of the stomach contents.

An incision was made along the ventral region of the mantle cavity to expose the internal organs. Following the recommendations of Li et al. (2018), a syringe was used to flush approximately 25 ml of distilled water over the mantle cavity, arms, funnel, gill lamellae, and egg strands. The resulting liquid was then passed through a 60 μm mesh disc; the mesh disc was then placed in a covered Petri dish to dry.

The materials retained on the mesh discs were visually inspected using a dissection microscope and any potential plastic particles were classified in terms of the fragment or fiber type, color, number, size, and a brief description; the materials were then photographed using a microscope-mounted camera (AxioCam ERc5s) in conjunction with a program for image analysis (Zen 2.3). The fractions of fibers of different size classes (< 0.25 , $0.25-0.50$, $0.60-1.00$, $1.10-5.0$, > 5.0 mm) were recorded. All the fibers were preserved for future polymers identification by Fourier transform infrared spectroscopy technique.

Data was tested for normality by Shapiro-Wilks test. Since the data

did not satisfy the supposition required to perform a parametric ANOVA, the Kruskal-Wallis non parametric test was used to test if there were any significant differences in the number of fibers among species.

To examine potential differences between the occurrences of fibers in stomachs with vs. without female *A. nouryi*, each species independently were analyzed using two-tailed Mann-Whitney *U* test (Zar, 1999). All statistical analysis was conducted using the software Statistica 7 (StatSoft, 2013).

3. Results

During 12 months of study, a total of 33 whole and undigested *A. nouryi* female were identified in the stomach contents of the large pelagic fish (Table 1). All female *A. nouryi* presented fibers adhering to the body, funnel, and mantle cavity (gill, intestine, ovary, oviducts). The number of fibers per specimen ranged from 5 to 293 with a mean of 67 (± 92); one Noury's argonaut contained 914 fibers (Fig. 2A); fibers were scarce on egg masses (< 15 fibers). The largest fiber measured > 5.0 mm (7.4%) and fibers ranged from of 0.25 to 5.0 mm in length (92.6%) (Fig. 2B). The most prevalent colors were hyaline (72.0%) (Figs. 2C and 3). We do not found fragments (Fig. 4).

The highest percentage of fibers (53.8%) adhering to *A. nouryi* were found in the stomach contents of *I. platypterus* (Fig. 2D). The Kruskal-Wallis test indicated statistically significant different for number of fiber per species ($\chi^2 = 4.02$, $df = 2$, $p < 0.05$). In the stomach contents without any whole Noury's argonauts, but did contain Argonaut beaks, we observed fibers (Table 1).

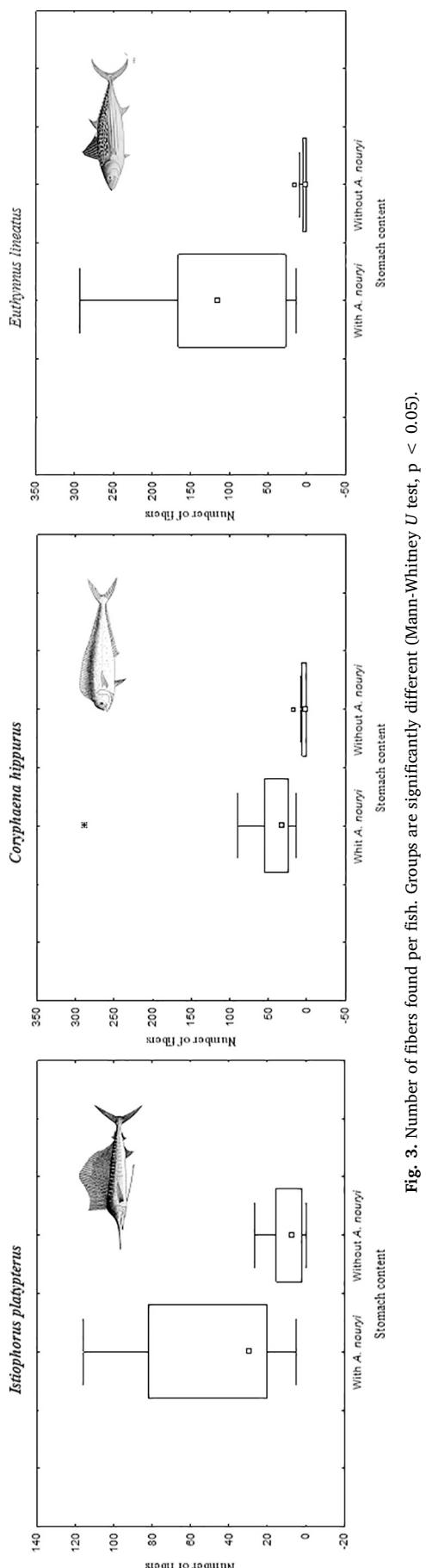


Fig. 3. Number of fibers found per fish. Groups are significantly different (Mann-Whitney U test, $p < 0.05$).

The concentration of fibers was significantly higher in stomachs with Noury's argonauts than those without: *E. lineatus* ($U = 1.0$, $p = 0.0004$), *I. platypterus* ($U = 19.5$, $p = 0.0004$), and *C. hippurus* ($U = 10.0$, $p = 0.0002$) (Fig. 3).

4. Discussion

This study is the first to describe the buoyant fibers adhering to the body and mantle cavity of *A. nouryi* females. All specimens examined as part of this research presented adhering fibers, suggesting that microplastics and textile fibers are common in the epipelagic environment. Egg masses are reared inside the female shell until juveniles are ready to hatch into plankton (Nesis, 1977). As with other octopuses (Villanueva and Norman, 2008), females continuously clean the egg surfaces with their suckers and ventilate the eggs with water flushes from their funnels; thus, potentially preventing the accumulation of buoyant fibers on egg masses.

The dominant fiber color was hyaline (72%), suggesting the fibers derive from common plastic objects made of polyethylene, polypropylene (i.e., beverage bottles and bags), and polyvinyl (i.e., parts of fishing floats and fishing line); textile industry remnants are another major source of fibers. Compa et al. (2018) reported that both microplastic and natural fibers of anthropogenic origin are common throughout the pelagic environment; according to Reisser et al. (2015), 41% of microplastics occur between 0.5 and 5 m deep.

Female Noury's argonauts appear in high numbers in coastal areas, forming long chains of up to 18 individuals (Rosa and Seibel, 2010); they are frequently found in the vicinity of floating objects (Nesis, 1977), both natural and artificial, such as logs, branches, ropes, buoys, and kelp (Rose and Hassler, 1974). Along the coast of Oaxaca, most of these floating objects are the remains of fishing gear, fishing floats, and fishing lines composed of multifilament polyethylene, land vegetation, and plastic debris (pers. and local fishermen obs.) transported by rivers and municipal drainage systems into the ocean; their abundance increases during the rainy season. In the study area, there are three permanent rivers that drain the Oaxaca highlands: Colotepec, Copalita, and Tehuantepec (Fig. 1); discharge and runoff from these rivers may be an important source of fibers from textiles, plastics, and other floating debris. Our results may reflect an increment in the concentration of buoyant fibers in surface waters, increasing the probability of fibers adhering to *A. nouryi*.

Likewise, large epipelagic fishes also tend to associate with floating objects (Rose and Hassler, 1974); their feeding on aggregated prey (bony fishes, squid, argonauts, and crustaceans) may increase their ingestion of fibers. *I. platypterus*, *C. hippurus*, and *E. lineatus* are opportunistic species with high consumption of *A. nouryi* (Alejo-Plata et al., 2019). Our results indicate that the fish species analyzed are subject to fiber ingestion; there were also significant differences between species, reflecting their distinct feeding strategies.

The amount of fibers in the stomach contents with Noury's argonauts was significantly higher than in stomachs without *A. nouryi*; this suggests that fibers may be introduced via this prey species. Our study is an important initial contribution; similar studies on other species will further improve our estimates of buoyant fiber concentration levels, size distributions, drift patterns, and interactions between neustonic and epipelagic species in the world's oceans.

Large predators are an important component of the epipelagic food web; as such, their feeding ecology offers important clues regarding the underlying ecosystem structure (Olson and Galván-Magana, 2002). However, plastic ingestion by large epipelagic fish species has not been investigated thoroughly (Romeo et al., 2015; Alomar and Deudero, 2017). Our results indicate that the analysis of the stomach contents of large predators could serve as a significant source of data for describing marine litter and fibers in the pelagic ecosystem.

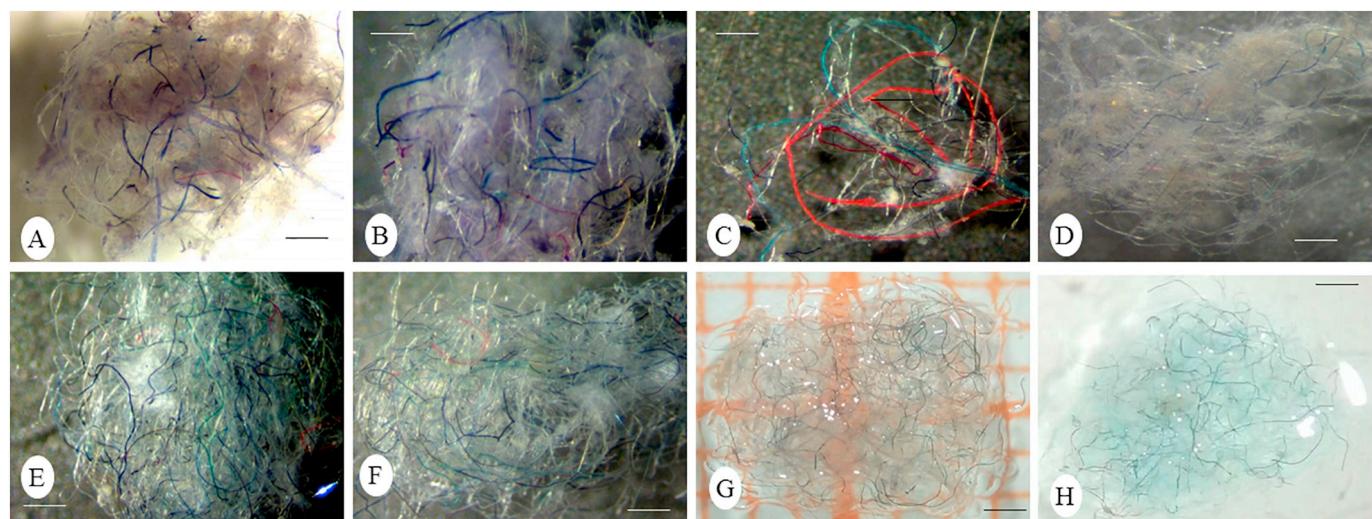


Fig. 4. Examples of fibers recovered in *A. nouryi* females. Pictures of some collected fibers are reported as representative of general features of fibers in this study. A–G, scale bars represent 1 mm; H scale bar represent 0.1 mm.

CRediT authorship contribution statement

María del Carmen Alejo-Plata: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft. **Eduardo Herrera-Galindo:** Investigation, Formal analysis, Methodology, Writing - review & editing. **Diana Guadalupe Cruz-González:** Formal analysis, Methodology, Writing - review & editing.

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