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First record of *Pickfordiateuthis vossi* Brackoniecki, 1996 (Myopsida, Loliginidae) early life stages in the central Mexican Pacific

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Abstract

The grass squid *Pickfordiateuthis vossi* Brakoniecki, 1996 is a dwarf species distributed along the northwest coast of Mexico. In the eastern Pacific, little is known about its distribution and life cycle. Two specimens, which are considered the smallest individuals of the genus collected to date, were caught in zooplankton trawls during 2 oceanographic cruises (January and March 1998) carried out in the central Mexican Pacific. The paralarval and juvenile stages are described and represent a new record in the area, with a range extension of 600 km southwest from the nearest previous record.

Key words

Cephalopod, grass squid, juvenile, loliginid, new record, paralarvae.

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Introduction

Squids of the Loliginidae family are morphologically characterized by a corneal membrane covering the eyes. Species can reach up to 900 mm in mantle length as in *Loligo forbesii* Steenstrup, 1856, but those of *Pickfordiateuthis* Voss, 1953 are dwarf species that measure up to 13 mm (Brakoniecki 1996, Jereb and Roper 2010). They exhibit a broad distribution in the neritic zone of subtropical, tropical and temperate seas all over the world (Sweeney et al. 1992). Seven loliginid species have been reported in the Mexican Pacific: *Doryteuthis opalescens* Berry, 1911; *Lolliguncula panamensis* Berry, 1911; *Lolliguncula argus* Brakoniecki & Roper, 1985; *Lolliguncula diomedeae* Hoyle, 1904; 2 additional forms recently recognized, *Lolliguncula* sp. 1, and *Lolliguncula* sp. 2 (Granados-Amores et al. 2014); and *Pickfordiateuthis vossi* Brakoniecki, 1996.

Species within the genus *Pickfordiateuthis* differ morphologically from other loliginid cephalopods and are characterized by having 2 rows of suckers on the manus of the tentacular club, with adults considered dwarf (Jereb and Roper 2010) due to their maximum mantle length of 20 mm, rendering them of no commercial importance. *Pickfordiateuthis vossi* is the only species of its genus that inhabits the Pacific Ocean, with

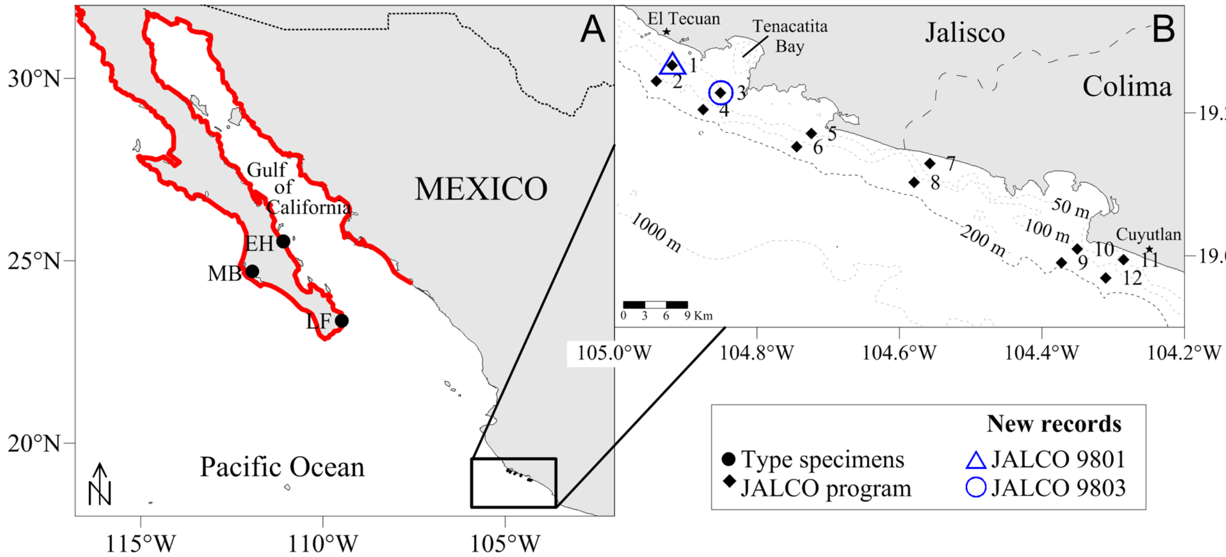

Figure 1. *Pickfordiateuthis vossi*. Current distribution of grass squid in the Pacific Ocean (Jereb and Roper 2010). Solid circles indicate location of holotype from Magdalena Bay (MB), and paratypes from Los Frailes (LF) and Escondido Harbor (EH) in the south of the Baja California peninsula, Mexico (**A**). Sampling grid stations of JALCO monitoring program with location of new records (**B**).

records off the west coast of the Baja California Peninsula and inside the Gulf of California, Mexico (Jereb and Roper 2010). *Pickfordiateuthis pulchella* Voss, 1953, *P. bayeri* Roper & Vecchione, 2001, and the morphotype *Pickfordiateuthis* sp. A occur in the Atlantic Ocean (Brakoniecki 1996). These species inhabit shallow waters, sea grass beds, and reefs, while distribution in deeper waters is probably related to reproductive migration (Roper and Vecchione 2001).

Almost nothing is known about the ecology, reproductive biology, spawning areas, and distribution of *P. vossi* along the coastal zone in the tropical Pacific. In addition, there is a complete lack of morphological descriptions of the early stages of this genus. Here we report for the first time the presence of *P. vossi* in the coastal zone of the central Mexican Pacific, provide a morphological description of specimens, evidence of reproductive activity of the species, and update its geographical distribution.

Methods

Study area. The Mexican central Pacific is characterized by transitional hydrographic conditions determined by the influence of the tropical Mexican Coastal Current (MCC), which flows northward, and the cooler and saltier California Current (CC), which flows towards the equator (Portela et al. 2016). Three hydrographic periods are found in this area: 1) mixed, with intense upwelling events that promote primary production from February to May; 2) stratified, with the dominance of tropical conditions from July to November; and 3) semi-mixed, during June and December, marked by the transition among relaxation and upwelling periods (Ambriz-Arreola et al. 2012, Gómez-Valdivia et al. 2015, López-Sandoval et al. 2009). During the first half of 1998, the region was under the influence of the 1997–1998 El Niño event; positive MEI (Multivariate El Niño Index) surface anomalies were highest in January, lasting until May (https://www.esrl.noaa.gov/psd/enso/mei/).

Field sampling. The specimens were obtained from zooplankton samples taken in January and March 1998 as part of the JALCO oceanographic monitoring program. This program surveyed a coastal fringe (12-station plan) along the continental shelf off southern Jalisco and Colima, Mexico, located between El Tecuán in Jalisco (19°18.5′ N, 104°56.2′ W) and Cuyutlán in Colima (18°58′ N, 104°14′ W) from 1996 to 1998 (Franco-Gordo et al. 2002) (Fig. 1). Zooplankton were sampled by oblique Bongo net tows (Smith and Richardson 1977) equipped with calibrated flowmeters, and following Kramer et al. (1972) to a maximum depth of 120 m. After each trawl, zooplankton samples were fixed in 4% formaldehyde buffered with sodium borate. In addition, temperature, salinity, and depth were measured to approximately 10 m above seafloor with a CTD (Seabird SB09). Surveys were carried out onboard the research vessel BIP-V, property of Departamento de Estudios para el Desarrollo Sustentable de la Zona Costera, Universidad de Guadalajara, and financed by the Universidad de Guadalajara and the Consejo Nacional de Ciencia y Tecnología (CONACYT). Surveys were done under permit no. 150995-214-03, which was issued by the Secretaría de Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP).

Laboratory procedures. In the laboratory, all cephalopod specimens were sorted from each zooplankton sample, identified to species (Brakoniecki 1996, Sweeney et al. 1992), and counted. Abundance was standardized to number of paralarvae per 1,000 m^3 of filtered water (PL/1000 m^3) (Postel et al. 2000). The 2 *Pickfordiateuthis* specimens found were analyzed separately. Counts and measurements (before preservation) were taken

Table 1. *Pickfordiateuthis vossi* abundance and environmental conditions of sampled sites. Min = minimum, Max = maximum.

Site	Geographic position		Abundance (PL/1000 m³)	Temperature (°C)			Salinity		
	Latitude	Longitude		Mean	Min	Max	Mean	Min	Max
9801-01	19°15.99′N	104°55.13′W	11	25.2	21.7	26.4	34.0	33.8	34.5
9803-03	19°13.70′N	104°51.07′W	11	23.3	16.3	25.9	34.3	34.1	35.1

following Roper and Voss (1983) with a calibrated ocular micrometer (precision 0.1 mm) adapted to a Carl Zeiss stereomicroscope Stemi 2000-C.

In descriptive systematics of cephalopods, morphological indices for comparing individuals of the same species or between different species are widely used. The primary index is a direct proportional relationship of the dorsal mantle length (ML), calculated by the following equation: [(character measurement/ML) × 100] (Roper and Voss 1983). Indices calculated, as percentages of the ML, were: MWI = mantle width index, HWI = head width index, FLI = fin length index, FWI = funnel width index, AL = arm length index for I, II, III, IV = arm I to IV respectively; HcLI = hectocotylus length index, TLI = tentacle length index; and CLI= tentacular club length index.

After identification and measurement of each character, voucher specimens were preserved in 96% ethanol and deposited in the zooplankton collection of the Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras (DEDSZC-ZMol) of Universidad de Guadalajara in San Patricio-Melaque, Jalisco, Mexico, with register numbers DEDSZC-ZMol-001 and -002.

Results

A total of 32 and 20 cephalopod paralarvae and juveniles (314 PL/1000 m^3 and 200 PL/1000 m^3) were collected during the January and March cruises respectively, from which 2 specimens of *Pickfordiateuthis vossi* were recorded. The distribution of temperature and salinity in both sampling stations was homogeneous in the entire water column. Mean temperature was 25.2 and 23.3 °C in sites 9801-01 and 9803-03, respectively, and mean salinity was 34 in site 9801-01, and 34.3 in site 9803-03 (Table 1).

Order Teuthida Naef, 1916
Suborder Myopsina Orbigny, 1841
Family Loliginidae Lesueur, 1821
Genus *Pickfordiateuthis* Voss, 1953

***Pickfordiateuthis vossi* Brakoniecki, 1996**

New records. Mexico: Jalisco: La Huerta: southwest Tenacatita Bay: Site 9801-01 (19°15.99′ N, 104°55.13′ W, 110 m depth), Carmen Franco-Gordo and Aramis Olivos-Ortiz, January 1998, 07:00 hrs (1 specimen, paralarvae, 4.0 mm ML, DEDSZC-ZMol-001). Mexico, Jalisco: La Huerta: southeast Tenacatita Bay: Site 9803-03 (19°13.70′ N, 104°51.07′ W, 74 m depth), Carmen Franco-Gordo and Enrique Godínez-Domínguez, March 1998, 20:00 hrs (1 specimen, juvenile, 6.8 mm ML, DEDSZC-ZMol-002).

Identification. The morphology of the 2 specimens examined agrees with *P. vossi* described by Brakoniecki (1996). Mantle cylindrical and stout with bluntly pointed posterior. Fins short, rounded, not united posteriorly. Funnel short and stout with straight mantle locking apparatus. Funnel organ consisting of an inverted U-shaped dorsal pad and a pair of ventral oval pads. Head square and width about half of the mantle length (Table 2). Arms short stout and well developed, and oral surface with 2 rows of suckers. Arms formula III > IV ≈ II > I. Tentacles are stout. Tentacular club is greatly elongated with no distinguishable carpus, manus, or dactylus. Both

Table 2. *Pickfordiateuthis vossi*. Morphological indices (%) in: *this study, **holotype and paratypes from Brakoniecki (1996). ML = mantle length (mm). Following indices are in percentage (%) of the ML. MWI = mantle width index, HWI = head width index, FLI = fin length index, FWI = funnel width index, AL = arm length index for I, II, III, IV = arm I to IV respectively, r = right, l = left, HcLI = hectocotylus length index, TLI = tentacle length index, and CLI = tentacular club length index.

Index	Arm	Specimen 1*	Specimen 2*	Males**	Females**
ML		4.0	6.8	10.5–14.6	12.9–18.4
MWI		63.5	48.0	40.2–55.2	34.2–52.8
HWI		55.0	41.6	34.3–45.8	31.0–42.4
FLI		32.8	31.9	27.0–31.7	27.8–33.3
FWI		35.5	30.3	49.2–70.5	53.5–63.2
ALI	I	22.5	31.3	19.9–26.7	13.0–27.2
	II	40.5	44.1	27.9–41.9	25.0–34.9
	III	53.0	55.1	34.6–46.7	27.7–40.5
	IVr	39.3	39.1	25.7–37.5	21.7–32.3
	IVl	44.0	39.1	27.9–41.9	26.2–33.3
HcLI		—	—	25.5–35.6	—
TLI		64.8	48.6	39.0–49.2	30.9–43.7
CLI		37.5	23.0	33.8–44.2	26.1–41.1

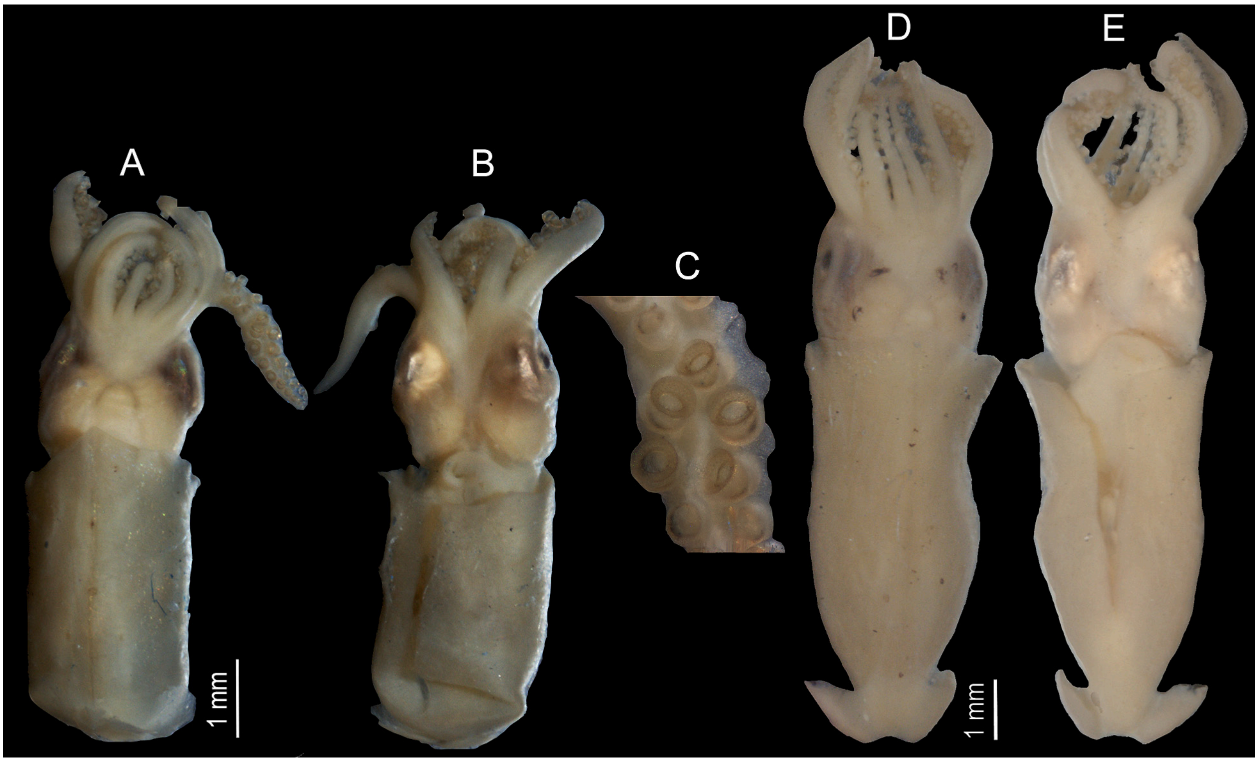

Figure 2. *Pickfordiateuthis vossi*. Dorsal (**A**) and ventral (**B**) view of 4.0 mm ML paralarvae and detail of tentacular club (**C**) with 2 rows of suckers. Dorsal (**D**) ad ventral (**E**) view of 6.8 ML juvenile.

specimens presented a biserial arrangement of suckers, distinctive for the species. Furthermore, the fact that *P. vossi* is the only species of its genus currently known in the Eastern Pacific, support our species identification.

Specimen 1, ML = 4 mm (Fig. 2A–C). Few chromatophores visible over entire body. Dorsal mantle surface with 4 chromatophores along the gladius. No visible chromatophores over fins, ventral mantle, nor on surface of funnel. Head without visible chromatophores on dorsal or ventral surface. Lobes of buccal membrane not developed, with no sucker on margin. Seminal receptacles not visible. Margin of sucker ring without developed teeth. Chromatophores not visible in aboral surface. Left ventral arm (IV) without modification. Tentacles stout and slightly larger than arm III. Tentacular club with 1 sucker in the proximal region followed by 2 rows of suckers until distal tip of tentacle. Proximal and middle suckers larger than distal ones. Sucker rings of tentacles with approximately 15–16 pointed teeth regularly spaced with those in the distal margin larger than the rest.

Specimen 2, ML = 6.83 mm (Fig. 2D, E). Dorsal mantle surface with 4 chromatophores in anterior zone, 4 in posterior half, and 2 on posterior margin. Fins with 1 chromatophore at base. Funnel surface with no visible chromatophores. Dorsal surface of head with 5 chromatophores in a pyramid shape. No visible chromatophores on ventral surface of head. Buccal membrane same as specimen 1 but with the presence of what appears to be a seminal receptacle. Sucker rings of arms with no teeth on margin. Tentacle sucker rings with 20 pointed teeth around margin with those on distal part larger than on proximal.

Discussion

Pickfordiateuthis vossi was recently described by Brakoniecki (1996), and existing reports have placed its distribution as restricted to the west coast and Gulf of California, Mexico (Jereb and Roper 2010).

The presence of *P. vossi* on the southern coast of Jalisco represents a range extension of approximately 600 km from the nearest previous record. This could be explained by the effect of warm water at the time of sampling of the 1997–1998 El Niño event, which could influence the distribution of this species as squids follow their optimum thermal preference (Pecl and Jackson 2008) or because the effect of the local mesoscale structures that transport the organisms from the coast to the open ocean (Godínez et al. 2010, Pelayo-Martínez et al. 2017). The effect of the same El Niño event was recognized for other loliginid species like *Doryteuthis opalescens*, which caused this species to move offshore the Southern California Bight (Zeidberg and Hamner 2002). Nevertheless, our new record could also be due to coastal sampling in a poorly sampled region as the Mexican central Pacific.

Adult specimens reported by Brakoniecki (1996) ranged from 10.5–14.6 mm ML in males, while females ranged from 12.9–18.4 mm ML. Accordingly, our specimens represent the smallest individuals (4.0 mm and 6.8 mm ML) collected to date (Table 1). Based on the definition of Young and Harman (1988), the end of the paralarvae stage is mainly followed by ecological or habitat change, but this is not always true for all species. In many cases, transition from paralarvae to subadult concern changes in body proportions, and changes in the structure or function of specific organs, as in the separation of the fused tentacles (proboscis) in ommastrephid rhynchoteuthion paralarvae (Robin et al. 2014, Shea and Vecchione 2010). In this sense, considering that adults of *P. vossi* are "dwarfs", specimen 1 could be a paralarva, indicating reproductive activity of the species in the area, and specimen 2 would be more likely a juvenile, measuring only 4 mm smaller than the smallest female reported (Brakoniecki 1996).

It is known that alcohol used in the preservation of zooplankton samples causes some dehydration to soft-bodied organisms. This is not the case in our materials, as JALCO zooplankton samples and therefore our specimens of *P. vossi* were fixed in 4% formaldehyde buffered with sodium borate, and measurements were taken before preservation in 96% ethanol. Also, Goto (2005) showed that changes in the mantle lengths of paralarvae fixed in borax-buffered formalin and preserved in 99% ethanol were not statistically significant. For this reason, the sizes of specimens from the central Mexican Pacific can be confidently considered the smallest recorded to the date.

Except for FLI, ALI, and CLI, the morphological proportions from the holotype and paratypes (Brakoniecki 1996) were noticeably different than the ones calculated from our specimens. FWI of specimens 1 and 2 were smaller than those from Brakoniecki (1996), but meanwhile the rest of the indices were greater. These differences in body proportions may be because this species presents negative allometric arm growth (II–IV) and head width narrowing as squids transition into subadulthood (Robin et al. 2014). Unlike Brakoniecki's (1996) report of 6 short blunt teeth on the distal margins of the arm sucker rings, our specimens had the margin of the sucker rings entirely smooth, but this is probably because teeth are likely to develop with growth as in other cephalopods (De Silva-Dávila 2013). Nevertheless, more studies are needed to complete the record of the morphological changes related to growth in the life cycle of this species.

The poor or non-pigmentation of the chromatophores observed on the body surface is owed to the extended time lapse between collection and analysis of the zooplankton samples and the use of formaldehyde solution for fixing and preserving, which usually fades the chromatophores, particularly in squids. The lack of chromatophores was also observed by Goto (2005) who mentioned that chromatophores of paralarvae fixed and preserved in buffered formalin showed translucent mantles after 3 months. Therefore, better observations of chromatophore patterns in recently collected specimens are required to gather a better description of the chromatophore patterns of this species at early stages.

The habitat the genus *Pickfordiateuthis* is described as sand and seagrass meadows or reefs (Jereb and Roper 2010), and the coastal area in front of Jalisco, where the Tenacatita coral reef is located, appears to be a suitable environment for reproduction and growth for *P. vossi*. Nevertheless, because of its tiny size and very coastal distribution, the catch of this species in common fishing gear or scientific monitoring is unlikely, and in consequence, knowledge of distribution and life cycle is scarce and particularly information on the paralarval and juvenile stages. To date, besides the 2 specimens found in this study, only 1 *Lolliguncula* specimen in early-life stage and none from *Pickfordiateuthis* have been reported in a region extending from the Gulf of California to Bahía de Banderas, probably because of the few coastal stations sampled (De Silva-Dávila et al. 2015). Granados-Amores et al. (2014), analyzed species of loliginids in the Mexican Pacific using specimens collected as bycatch in shrimp trawls and did not capture any specimen of *P. vossi*. Similar difficulties in the collection of specimens occur with other pickfordiateuthids, as is the case for the Atlantic species *P. pulchella*, of which Araujo and Gasalla (2018) only found a single specimen out of 246 quantified off the South Brazil Bight. Consequently, we recommend that more coastal surveys be incorporated into regional zooplankton sampling programs to improve the knowledge of cephalopod early life stages diversity in the area, particularly of the genus *Pickfordiateuthis*, considering that at the time of our sampling the area was under the influence of a strong El Niño event.

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Authors' Contributions

CFG and AOO collected the zooplankton samples and separated cephalopod specimens during oceanographic cruises. GPM and RSD identified the specimens and made the figures. All authors contributed to the writing of the manuscript.

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