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Symbiotic relationship between *udonella* sp. (monogenea) and caligus rogercresseyi (copepoda), a parasite of the chilean rock cod *eleginops maclovinus* 

Relación simbiótica entre *udonella* sp. (monogenea) y *caligus rogercresseyi* (copepoda), parásito del pez *eleginops maclovinus* en Chile

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# **SUMMARY**

This paper studies the host-parasite relationship between the worm *Udonella* sp. (Monogenea) found on the genital segment of the sea lice *Caligus rogercresseyi* (Copepoda), a common parasite of the rock cod *Eleginops maclovinus* found off the coast of southern Chile. The biological association between these invertebrates is interesting because *C. rogercresseyi* also infects farmed salmon (Boxshall and Bravo, 2000) and eventually *Udonella* sp. could be used for the biological control of

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sea lice. Rock cod were captured with hook and line and examined in the laboratory for lice, selecting only those C. rogercresseyi ovigerous females with Udonella sp. A video camera placed on a stereomicroscope was used for the in vitro observation of worms on the copepods on which they survive. Egg strings of copepod females with *Udonella* were later fixed in buffered formalin at 5% and stained with hematoxylin-eosin and prepared for histological sections of the structures involved in host-parasite attachment. For histochemical analysis toluidine blue at different pH levels was used. Observations with hematoxilin \_ eosin showed that the external cuticle of the egg sac is acidofilic and remains undamaged while Udonella adhesive disc is attached. When toluidine blue was used, the attachment area showed slight basophylic stains and metachromasia visible at pH of 1.2. Udonella at the caudal gland level as well as C. rogercresseyi at the sub-cuticular region of the egg sacs showed basophylic reaction at pH of 4.5. The present paper clarify the absence of possible mechanical damage in the parasite-host attachment site and provides an histological description of the wall dependent contact points between Udonella - Caligus plus the extracellular space that separates them. Udonella survived on mucus secreted by the rock cod's skin and not on the content of the copepod's egg sacs, which serve more as an attachment area given that in this zone what is seen is a metachromatic stained strip corresponding to acid glycosaminoglycans (Aranishi & Nakane, 1998; Buchmann, 1998). The association between the worm and the copepod is commensalistic.

Key word: Udonella, Caligus rogercresseyi, symbiosis, salmonids.

Palabras claves: Udonella, Caligus rogercresseyi, simbiosis, salmones.

#### INTRODUCTION

The genus Udonella (Platyhelminthes: Udonellidae) is of particular interest to parasitologists because its members are symbionts on copopods, that are parasites of marine fish. The systematic position of *Udonella* has been controversial, and has recently been placed among the monogeneans (Littlewood et al., 1998). Prior to the advent of synthetic chemical pesticides, the use of natural compounds and natural enemies was an important area of applied research in parasite control, particularly in aquaculture. The environmental damage caused by pesticides and the development of genetic resistance by pests has renewed the interest in biological control. The introduction of parasites in marine environments is common and damaging (Scholz, 1999) but pest control efforts in Chile have been limited to the use of conventional pesticides (e.g. nuvan and dichlorvos), (Sievers et al. (1995). Recent studies indicate that ballast water is the most important means of pest dissemination in marine systems (Lafferty and Kuris, 1996). A recent application of biological control in the marine environmment has been the use of wrasses (Labridae) to control in salmon lice, Lepeophtheirus salmonis in Europe (Treasurer and Costello, 1996). A biological system occurs in Chile composed of native rock cod Eleginops maclovinus that commonly are found around salmon pens; Caligus rogercresseyi (Copepoda: Caligidae) sea lice found as ectoparasites on rock cod and the monogenean Udonella sp. found on the copepod's body. The biological association between these invertebrates is interesting because C. rogercresseyi also infects farmed salmon (Boxshall and Bravo, 2000) and eventually could be used for the biological control of sea lice. Furthermore, this composition of organisms conforms to the biological control flow chart proposed by Lafferty and Kuris (1996) because there is a pest, and the existence of a natural enemy of the pest. The purpose of the present study was to clarify whether a mechanical and/or histological dammage at the attachment sites of *Udonella* could affect the integrity of the sea lice eggs.

### MATERIALS AND METHODS

Twenty ovigerous *C. rogercresseyi* infected with *Udonella* sp. were collected from rock cod *E. maclovinus* caught by hook and line in fish pens at aquaculture sites near Puerto Montt, Chile during 1998. Copepods and their symbionts were anesthesized with BZ 20 (benzocaine at 2%), subsequently fixed in a 5% formalin and filtered sea water solution, dehydrated and embedded, sagittally sectioned (8 mm) and stained with hematoxylin and eosin and toluidin blue at different pH levels (1.2, 2.5 and 4.5) in order to histochemically study the structure of the copepod worm interface. Live copepods representing different stages of development were used for behavioural

observations. These were placed in 9 cm diameter x 1 cm deep glass Petri dishes containing sea water. Many of the copepods were infected with *Udonella* sp., a stereomicroscope with a video camera connected to a TV monitor was used to observe worms movements on copepods and images were recorded on video cassette. Measurements are in mm unless otherwise indicated. Observations are centred around the attachment area between parasite and symbiont in order to see whether any mechanical or physiological effect exists that could damage the viability of the copepod eggs inside the ovigerous sac.

### **RESULTS**

Udonella sp. was tipically found on the genital complex and the egg sacs of the copepods, however exact attachment location depended on the worm's developmental stage. Eggs were attached to the posterior section of the genital complex, and juveniles and adults attached on the egg sacs by means of their posterior attachment disc (Fig. 1A). When Udonella sp. was in situ on a copepod, the worm sucked mucus from the environment everting and retracting its pharynx. Juvenile worms remained fixed and in clusters on the genital complex or alone on the egg sac. In vitro, adult worms firmly attached to the cuticle of egg sacs and they sometimes extended as if in search of food. The worm's pharynx worked actively up to the point of attaching itself to the egg sac, later releasing the posterior attachment disc and holding on by the pharynx. The attachment disc is later placed where the pharynx had been previously attached. It never leaves the body surface of the copepod. The symbiotic relationship between Udonella and Caligus does not alter the general morphology of either platyhelmint or copepod. The worm-copepod interface is composed by: a) Caligus ovigerous sac wall ; b) extracellular space; c) Udonella's attachment disc tegument (Fig 1B and C). The material stained with hematoxilin \_ eosin showed that the external cuticle of the egg sac wall in which Udonella's adhesive disc is attached is acidophilic and remains undamaged. A band of the same width as the cuticle, exists immediately below showing less density and acidophilia. The extracellular space is an interface in contact with the external environment and contains an homogenous mucus, varying in size according to the degree of adherence of each species. The tegument of the monogenean's adhesive disc on the contact wall, showed an intense basophilia consisting of the wrinkled surface and its numerous microvilli. Immediately over the area there is a membranous space into which numerous different size vesicles filled with a clear substance ended. These vesicles originate in the caudal gland and laterally surround the attachment disc. A thick acidophilic tegument with festooned borders was present. (Fig 1B). In order to observe the reactivity and to know the histochemical nature of different mucus like material, mainly the contents of the caudal gland and the coats, a differential stain using toluidine blue at pH 1.2, 2.5 and 4.5 which allowed the release of chemical groups belonging to the glycoproteins or mucus substances expressing diverse degrees of basophilia was applied. The egg sac cuticle did not react with the stain, but the area immediately below presented a graded staining intensity as the stain's pH increased. The interface was basophilic at pH 4.5 and showed metachromasia at pH 1.2, that may have indicated the presence of sulphate glycosaminoglycans (Fig. 1C). Reactive basophilia was only seen on the surface of Udonella sp. at pH 4.5 in the caudal gland area, and it was of the same intensity as what seen beneath the egg sac cuticle.

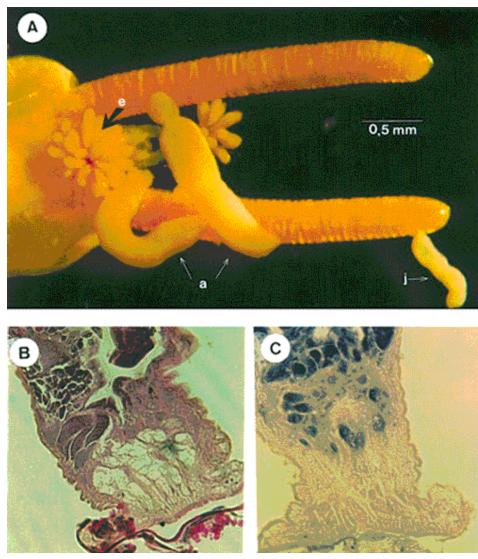


Figure 1. Udonella sp. on genital complex and egg sacs of Caligus rogercresseyi. Attachment locations of eggs (e) juveniles (j) and adults (a) of Udonella sp. (A). Histological sections in the adhesion area of attachment disc of worms to Caligus rogercresseyi egg sacs: hematoxylin and eosin (B), toluidine blue (C). 250x.

Udonella sp. sobre el complejo genital y sacos ovígeros de Caligus rogercresseyi. Lugares de fijación de los huevos (e), juveniles (j) y adultos (a) de Udonella sp. (A). Cortes histológicos en el lugar de adhesión del disco de fijación del helminto a los sacos ovígeros de Caligus rogercresseyi: hematoxilina \_ eosina (B), azul de toluidina (C). 250x.

# DISCUSSION

The copepod *Caligus rogercresseyi* has recently been described by <u>Boxshall and Bravo (2000)</u> from net pen reared salmonids in Chile and the monogenean *Udonella* sp. is also a new species, which is being described elsewere. The prevalence and abundance of *Udonella* sp. on copepods belonging to the genera *Caligus* and *Lepeophtheirus* was studied by <u>Carvajal et al. (1998)</u>. This study also evaluate fecundity, hatching and viability of these copepods on natives hosts. The worm and copepod parasite association is commensalistic, and at first sight seems to only function on native

benthic fish that differ from salmonids distributed on the upper portion of the water column. *Udonella* sp. develops entirely attached on the host in comparison to other monogeneans with mobile oncomiracidia larvae. The worm is attached to the copepod throughout the egg, juvenile and adult stages, but transfers of worms may take place during contact between the copepods while on the fish (Minchin and Jackson, 1993). Platyhelminth's in vitro feeding behaviour is explained since in the absence of rock cod, the monogenean takes in through the farynx the mucus present in the copepod's egg sacs. This should not be normal behaviour for *Udonella* sp., given that it depend on copepods for feeding on *E. maclovinus* mucus. Due to the attachment to the copepod's cuticle, the symbiont by itself could not elicit a mucus reaction from the rock cods' skin, but in association with *Caligus*, who feeds by pressing its tubular mouth on the host's skin and scraping its tissue through sucking with its mouth apparatus (Boxshall, 1990), a mucus reaction is produced on the fish skin where the parasite is at work (Dawson et al., 1997). It is on this fish mucus that *Udonella* sp. feeds. In the case of salmonids the same species of copepod parasite has been found, but never in association with the monogeneans. This could be explained by the fact that the characteristics of salmon mucus are not adequate for *Udonella* sp. to feed.

In general the mucous coats made up of mucoproteins, must be indicators of reaction specificity that allows external immunocompetition of the fish as well as its parasite. It is interesting to point out that the zone of attachment of the platyhelminth to the copopode is the only area where a metachromatic stain band appears, corresponding to acid glycosaminoglycans that could specifically be associated with membrane receptors that may be the immunochemical sign of the presence of simbionts (<u>Aranishi and Nakane, 1998</u>; <u>Buchmann, 1998</u>). This histological study reveal that *Udonella* sp. produce no damage to the egg sacs of *Caligus rogercresseyi*.

# **RESUMEN**

Se estudia la relación parásito - huésped entre el platelminto monogeneo Udonella sp. que vive sobre el segmento genital del piojo marino Caligus rogercresseyi parásito común del robalo Eleginops maclovinus en el sur de Chile. Esta asociación biológica es interesante de analizar, pues C. rogercresseyi también parasita a los salmónidos de cultivo y podría eventualmente Udonella ser utilizado como control biológico para el piojo del salmón. Los robalos fueron capturados con anzuelo y examinados por piojos en el laboratorio, seleccionándose los copépodos caligidos que presentaban Udonella sp. Posteriormente se sometieron estos helmintos a observaciones in vitro para estudiar la conducta que presentan sobre los copépodos en que viven, utilizando una cámara de video instalada en la lupa binocular. Luego, las udonellas que estaban sobre los sacos ovígeros de Caligus (cada saco usualmente llevaba de 3 a 4 helmintos) fueron fijadas en formalina tamponada al 5% y preparadas para cortes sagitales de las estructuras involucradas en la adhesión parásito - huésped, teñidas con hematoxilinaeosina y azul de toluidina. Con hematoxilina-eosina se revela la adhesión en la interfase dada por las microvellosidades membranosas de *Udonella* y la cutícula de *Caliqus* ligeramente acidófila, incluso en el punto de adhesión. La glándula caudal de Udonella muestra una conformación vesicular. Histoquímicamente, con azul de toluidina, la zona de la adhesión presenta una leve tinción basófila que se revela metacromática a pH 1.2. La superficie de Udonella a nivel de la glándula caudal, al igual que la sub-cutícula de los sacos ovígeros de C. rogercresseyi presenta basofilia reactiva sólo a ph 4.5. Udonella se alimenta del mucus secretado por la piel del robalo y no del contenido de los sacos ovígeros del copépodo, que mas bien les sirven como zona de fijación, dado que en la zona de adhesión aparece una banda de tinción metacromática que corresponde a glicosamino-glicanos ácidos que pudieran asociarse específicamente a receptores de membranas y ser la señal inmunoquímica de reconocimiento a la presencia del hipersimbionte. Se concluye que Udonella sp. no altera las cubiertas ni el contenido de los sacos ovígeros de C. rogercresseyi, por lo cual no sería factible utilizar este comensal para el control biológico de la parasitosis por Caligus en los salmones. La asociación biológica entre estos invertebrados es un comensalismo.

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