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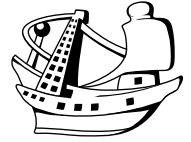


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## Morphological and molecular characterization of *Perinereis gualpensis* (Polychaeta: Nereididae) and its phylogenetic relationships with other species of the genus off the Chilean coast, Southeast Pacific

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**Abstract:** The genus *Perinereis*, family Nereididae, consists of approximately 66 worldwide distributed species with marine, estuarine, and freshwater representatives. The objective of this study is to use morphological and molecular traits to evaluate the taxonomic differentiation of the estuarine polychaete *P. gualpensis*. Also we reconstruct the phylogenetic relationships between *P. gualpensis* and congeneric species recorded in Chilean coast: *P. falklandica*, *P. longidonta* and, especially with the morphologically most similar species, *P. vallata*. Phylogenetic analyses based on molecular and morphological characters allow us to validate *P. gualpensis* and *P. vallata* as two independent and clearly defined species. Consequently, the taxonomic confusion about the possible sibling species *P. gualpensis* and *P. vallata* is completely resolved. However, morphological and genetic data are partially incongruent about the phylogenetic relationships of these four *Perinereis* species. We concluded that different evolutionary process that affect the morphological and genetics data produce different answers. Also we propose the use of new molecular evidence arising from molecular markers with different nucleotide substitution rate to that used here.

**Résumé :** Caractérisation morphologique et moléculaire de *Perinereis gualpensis* (Polychaeta : Nereididae) et de ses relations phylogénétiques avec d'autres espèces du genre sur les côtes chiliennes, sud-est de l'Océan Pacifique. Le genre *Perinereis*, famille Nereididae, comprend environ 66 espèces de distribution mondiale, avec des représentants marins, estuariens et d'eau douce. L'objectif de cette étude est d'utiliser des caractères morphologiques et moléculaires pour évaluer la différenciation taxonomique chez le polychète estuarien *P. gualpensis*. Nous avons estimé les relations phylogénétiques entre *P. gualpensis* et celles congénères enregistrées dans les environnements côtiers du Chili : *P. falklandica*, *P. longidonta* et surtout, avec l'espèce la plus morphologiquement similaire *P. vallata*. Les analyses phylogénétiques basées sur les caractères morphologiques nous permettent valider *P. gualpensis* et *P. vallata* comme deux espèces indépendantes et clairement définies. Par conséquent, la confusion taxonomique parmi les possibles espèces sibling *P. gualpensis* et *P. vallata* est complètement résolu. Toutefois, les données morphologiques et moléculaires sont parcellément incongrues pour les relations phylogénétiques entre les quatre espèces de *Perinereis*. Nous avons conclu que les différents processus

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d'évolution qui influent sur les données morphologiques et génétiques produisent, en conséquence, des réponses différentes. On propose également l'utilisation de nouveaux tests moléculaires dérivés de marqueurs moléculaires avec un taux de substitution de nucléotides différent de ceux utilisés ici.

**Keywords:** Cytochrome oxidase I • Morphology • Paragnaths • *Perinereis* • Phylogeny • Taxonomy

## Introduction

The genus *Perinereis* Kinberg, 1865, family Nereididae, consists of approximately 66 worldwide distributed known species. This genus is mainly recognized by the presence of elongated bar-shaped paragnaths in Area VI of the proboscis, which could be either compact with a distal pointed tip or forming a transverse row of small bars (Rozbaczylo & Castilla, 1973). Other important characters to define the genus are notochaeta with spinigers only and falcigers absent, and neurochaeta with heterogomph and homogomph spinigers and heterogomph falcigers (Bakken & Wilson, 2005; Santos et al., 2005). Studies on *Perinereis* genus are mostly related to life cycle (Prevedelli & Cassai, 2001), and taxonomic and systematic aspects (e.g. Hutchings et al., 1991; Wilson & Glasby, 1993; de León-González & Solís-Weiss, 1998). Studies using molecular data have been principally focused on population differentiation (Rouabah & Scaps, 2003) and species delimitation (Chen et al., 2002; Park & Kim, 2007).

Among the *Perinereis* species, nine have been reported from the Chilean coast, Southeast Pacific. *P. anderssoni* (Kinberg, 1866); *P. helleri* (Grube, 1878); *P. singaporiensis* (Grube, 1878); *P. vallata* (Grube, 1858); *P. falklandica* (Ramsay, 1914); *P. camiguinoides* (Augener, 1922); *P. pseudocamiguina* (Augener, 1922); *P. gualpensis* (Jeldes, 1963) and *P. longidonta* (Rozbaczylo & Castilla, 1973). *P. falklandica*, *P. longidonta*, *P. vallata* and *P. gualpensis* have been recorded in coastal environments, while *P. camiguinoides*, *P. helleri*, *P. singaporiensis* and *P. anderssoni* have only been recorded in insular environments (Juan Fernández Islands and Easter Island; Rozbaczylo, 1985). This diversity has only been studied by two studies that consider detailed descriptions of the *Perinereis* species from the Southeast Pacific. (1) Rozbaczylo & Castilla (1973) established the main morphological traits for the recognition of the genus. As a consequence, these authors provided a key to seven *Perinereis* species, although there is no description of *P. gualpensis*; (2) Bertrán (1980) focused on the taxonomic analysis of *P. gualpensis* and *P. vallata*. However, their diagnostic characters were not well defined, and this author does not present a phylogenetic analysis to

support the description of these species. The lack of detailed systematic studies entails a difficult species differentiation. This taxonomic confusion is not unusual in *Perinereis* species, and the actual solution is the informal denomination of species complex, such as *Perinereis cultrifera* (Grube, 1840) (Scaps et al., 2000), or the *Perinereis nuntia* species group (Wilson & Glasby, 1993; Glasby & Hsieh, 2006). In the latter species group are included *P. vallata* and *P. gualpensis* (Glasby & Hsieh, 2006). According to Rozbaczylo & Castilla (1973), these species have been defined with unclear morphological characters which consequently produce taxonomic confusion. These authors propose that even could be a single species. Alternatively, Bertrán (1980) established that *P. vallata* and *P. gualpensis* present morphological and ecological differences enough to consider them as different species. Then, conclusions regarding to delimitation of these species have been qualitatively established, without formal quantitative analyses.

In this study, we evaluate the phylogenetic relationships between *Perinereis* species from the Chilean coast, Southeast Pacific. Our aim is to use morphological and molecular traits to evaluate the taxonomic differentiation of the estuarine polychaete *P. gualpensis*, and also to update the database of morphological and genetic traits available from *P. gualpensis*, *P. vallata*, *P. falklandica* and *P. longidonta*.

## Materials and Methods

### Data collection

Adult individuals of *Perinereis* and *Pseudonereis variegata* (Grube, 1857) were collected in different locations from May 2006 to April 2007. Individuals collected were maintained in seawater and subsequently fixed in 95% ethanol. For the morphological characterization, we recorded number of segments, length and width body measures; the numbers in parentheses represent the range among specimens. In addition, numbers of paragnaths were also counted for 8 Areas (I, II, III, IV, V, VI, and VII-VIII). Part of this material was preserved for the molecular study.

We choose *Pseudonereis variegata* as outgroup based on previous morphological phylogenetic studies on Nereididae. These works have shown close relationships between *Perinereis*, *Neanthes* and *Pseudonereis* genera (Bakken & Wilson, 2005; Santos et al., 2005; Bakken, 2007).

#### *Morphological database*

We obtain a morphological characters matrix from the characterizations available in the literature and direct examinations of individuals. The phylogenetic informative characters were chosen for phylogenetic analyses. Character data was treated as binary traits (i.e. presence and absence codification) considering missing-data.

#### *Morphological phylogenetic analyses*

These analyses were conducted using two approaches: Maximum Parsimony (hereafter MP) and Markov and Monte Carlo Chain method within a Bayesian framework (hereafter BMCMC). MP was run in PAUP\* 4.0B10 software (Swofford, 2003), with the data treated as unordered and equal weight, with a heuristic search implemented with a TBR search algorithm with 100 replicates. A bootstrap analysis with 10,000 replicates was performed to evaluate node support. To determinate the apomorphies and homoplasies and determine the characters diagnostic specifics we mapped each traits and state along the tree using Winclada software (Nixon, 1999). The phylogenetic reconstruction by means of BMCMC (Pagel & Meade, 2004) was carried out using the *BayesPhylogenies* program (available at <http://www.evolution.reading.ac.uk/BayesPhy.html>). The morphologic bayesian tree was determined using a time-non-reversible morphological model with a M2P parameter in which character gains or losses are treated with a different probability of occurrence. Every 1,000 trees were randomly sampled to ensure the samples independence. Trees sampled before the convergence zone of the Markov Chain (hereafter CZ) were burning. Finally, a majority-rule consensus tree was generated to determine the posterior probability distribution of the nodes occurrence.

#### *DNA extraction, amplification and alignment*

The DNA extraction was carried out using a modified salting-out method (Miller et al., 1988). For each individual, the nucleotide sequences of the mitochondrial cytochrome oxidase subunit I (COI mtDNA) were amplified by means of polymerase chain reaction (PCR). The PCR reaction was carried out in a reaction volume of 30  $\mu$ L, each with 21.85  $\mu$ L of ddH<sub>2</sub>O, 3.0  $\mu$ L of buffer 10X, 1.8  $\mu$ L MgCl<sub>2</sub> 50mM, 1.5 dNTPs 2.5mM, 0.3  $\mu$ L of each primer and 0.25  $\mu$ L of Taq 5u/ $\mu$ L (Biotools®). The amplification was carried out in a MJ Research® PT-200 thermal cycler with annealing at 49°C for 55 seconds. The

PCR product was purified using a QIAGEN® Purification kit and subsequently sequenced in both directions (forward and reverse) using universal primers for this marker developed by Folmer et al. (1994). The obtained sequences were previously edited before being aligned using the Clustal W program, implemented in BioEdit version 7.0.5.2 (Hall, 1999). 15 total sequences with approximately 700 base pairs per sequence were obtained: two for *P. falklandica* (Number Accession Genbank HQ705184, HQ705185), four for *P. gualpensis* (HQ705186, HQ705187, HQ705188, HQ705189), two for *P. longidonta* (HQ705190, HQ705191), five for *P. vallata* (HQ705192, HQ705193, HQ705194, HQ705195, HQ705196) and three for the outgroup *P. variegata* (HQ705183, HQ705197).

#### *Molecular phylogenetic analyses*

The MP analysis was performed using PAUP\* 4.0B10 software (Swofford, 2003), with the same considerations as for the morphological phylogenetic analyses. For the BMCMC analyses, the sequence evolution model that best explained the data was determined using the general likelihood-based mixture model (MM) by Pagel & Meade (2004). Different likelihood values for each model were obtained using a simple GTR matrix, followed by a GTR matrix plus heterogeneity rate with gamma distribution (1GTR+G) to a more complex matrix 5GTR+G. The subsequent analyses were conducted only with the matrix that MM model significantly increased the likelihood. In the selected model, the total number of iterations was 100.000,000, with a total of 9,000 trees, from which the first 100.000 obtained before reaching the convergence zone (CZ) were excluded. Then, we used these trees to calculate the majority rule consensus tree, and the consistency of the nodes with their posterior probability of occurrence.

## Results

### Taxonomic characterization

#### *Perinereis* Kinberg, 1865

*Perinereis* Kinberg, 1865: 175; Fauchald, 1977: 90; Hutchings et al., 1991: 245; Bakken & Wilson, 2005: 531.

#### *Type species*

*Perinereis novaehollandiae* Kinberg, 1865 by original designation.

#### *Diagnosis*

Prostomium with entire anterior margin, one pair of antennae, one pair of biarticulated palps with conical palpostyles. Peristomium with 4 pairs of tentacular cirri with distinct cirrophores. One apodous peristomial

segment, greater than the length of chaetiger 1. Maxillary ring of proboscis with conical paragnaths: Areas I-II, present or absent; Area III present; Area IV, present or absent, smooth bar-shaped, present or absent. Oral ring with conical paragnaths; Area V, present or absent; Area VI, present or absent, with smooth transverse bars present; Area VII-VIII present. First two pairs of the parapodia are uniramous. Dorsal notopodial ligule on anterior chaetigers equal to dorsal neuropodial ligule. Prechaetal notopodial lobe present or absent, smaller than dorsal notopodial ligule on anterior chaetigers, usually reduced or absent in the posterior chaetigers. Dorsal cirrus basally attached to dorsal notopodial ligule or mid-dorsally to subterminally attached on posterior chaetigers, lacking basal cirrophore. Postchaetal notopodial lobe present or absent. Notoacacula absent from the first two pairs of chaetigers. Notochaetae spinigers present. Neurochaetae with homogomph and heterogomph spinigers and falcigers present or absent.

#### Observations

The presence of smooth bar-shaped paragnaths in Area VI is diagnostic for the genus. In some specimens of *Perinereis suluana* and *Perinereis aibuhitensis*, the bars in Area VI are extremely short that can be confused with cones (Hutchings et al., 1991); however, most specimens have short bars in Area VI. Bar-shaped paragnaths in Area IV are only found in some species.

#### *Perinereis falklandica* (Ramsay, 1914)

*Nereis (Perinereis) falklandica* Ramsay, 1914: 44-46, pl. 3, figs.3-10

*Perinereis falklandica* Fauvel, 1941: 280-281; Hartman, 1953: 29; Day, 1954: 18; Wesenberg-Lund, 1962: 80-83, figs. 30-31; Hartmann-Schröder, 1962a: 410-411; 1965: 298-299; Rozbaczylo & Castilla, 1973: 218-220, figs.2 a-f; Rozbaczylo & Bolados, 1980: 214-216.

#### Material examined

Cocholgué beach, Concepción (36°35'41''S-72°58'41''W), 15 ind.; Tumbes beach, Concepción (36°38'02''S-73°05'28''W), 19 ind.; Pichicuyín beach, Valdivia (39°25'26''S-73°13'04''W), 12 ind.; Calfuco beach, Valdivia (39°47'34''S-73°23'32''W), 7 ind.; Mehuin, Valdivia (39°25'27''S-73°13'03''W), 8 ind.

#### Diagnosis

Length 38.30 mm (22.0-72.0), width excluding parapodia 8.8 mm (1.2-5.0); number of chaetigers 74 (48-188). The basal region of the prostomium is broad with a light mark in the medial basal area. Two antennae shorter than the length of the palps. Two pair of violet-colored eyes, poorly

distinguishable in some individuals. Two well-differentiated palps with conical palpostyles. Four pairs of smooth tentacular cirri, the longest pair extending back to chaetigers 2 to 5. Black robust jaws with 8-12 small blunt teeth, the distal part presents a bifid-point with a cutting edge. The paragnaths of the proboscis (Fig. 1A-B) are arranged as follows: Area I = highly variable with 18-226 forming a triangular area of small cones with one or two larger cones in the center; Area II = 13-43 cones irregularly arranged forming a semicircular patch; Area III = 6-28 cones arranged in two to three rows, but in some individuals forming an oval; Area IV = 19-43 large cones forming an irregular patch; Area V = 1-3 cones, although most commonly only one; Area VI = 1 paragnath, broad, transversely elongated, ending in a pointed-tip; Area VII-VIII = 110-285 small and large cones irregularly arranged in a broad band. The body width is quite uniform throughout its extension, abruptly tapering towards the posterior (last 10-12 segments) ending in a rounded pygidium with a ventral pair of long, smooth cirri. Parapodia of the anterior segments (Fig. 1C) with short, rounded lobes; the superior notopodial lobe is slightly longer than the inferior. In the posterior segments, the superior notopodial lobe is considerably elongated with the cirrus almost at the distal end (Fig. 1D). Notochaetae: homogomph spinigers only (Fig. 1E). Neurochaetae, dorsal fascicle: homogomph spinigers (Fig. 1F) and heterogomph falcigers (Fig. 1G). Neurochaetae, ventral fascicle; heterogomph falcigers.

Live individuals are dark greenish-brown. In some specimens this pattern is lighter towards posterior, with lines similar to fingerprints. *Perinereis falklandica* is quite iridescent, keeping this characteristic even after being preserved in alcohol.

#### Habitat

Marine rocky shore, associated with mussel bed.

#### Distribution

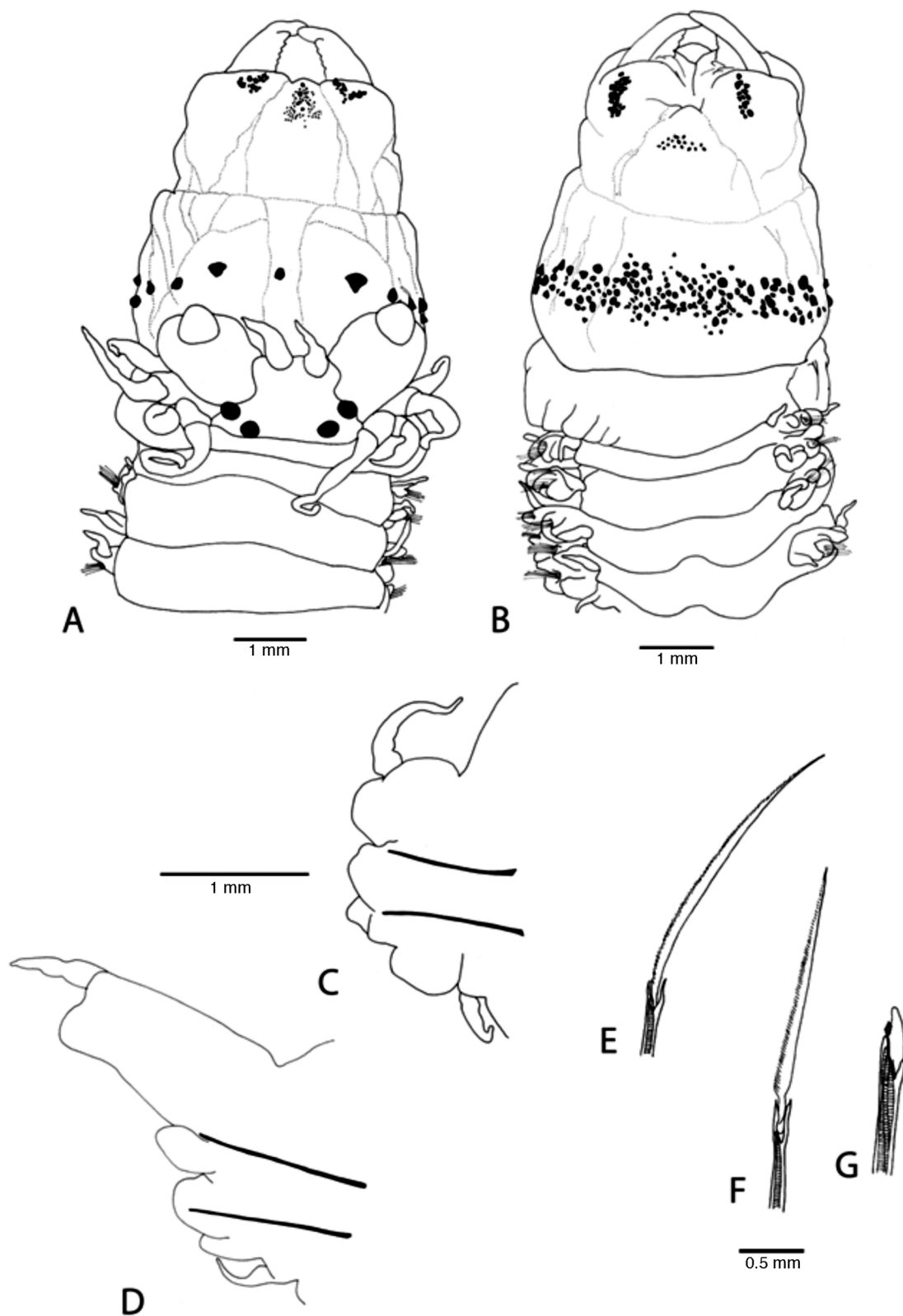
Falkland (Malvinas) Islands; Tristán da Cunha Island; Chile, from Arica to Cape Horn (Rozbaczylo & Bolados, 1980).

#### *Perinereis gualpensis* (Jeldes, 1963)

*Perinereis gualpensis* Jeldes, 1963: 4-9, figs. 2-5; Hartman, 1965: 38; Rozbaczylo & Castilla, 1973: 220; Bertrán, 1980: 82-83, fig. 1; Wilson & Glasby, 1993: 262-263.

#### Material examined

Biobío River mouth (36°S-73°W), 180 ind.; Itata River estuary (36°23'42''S-72°52'12''W), 33 ind.; Lingue River estuary (39°26'19''S-73°11'50''W), 40 ind.; Cutipay River estuary (39°51'16''S-73°19'58''W), 48 ind.; Queule River



**Figure 1.** *Perinereis falklandica* (Ramsay, 1914). **A.** Anterior end, dorsal view. **B.** Anterior end, ventral view. **C.** Parapodium 10th. **D.** Parapodium 60th. **E.** Notopodial homogomph spiniger chaeta, 10th chaetiger. **F.** Neuropodial homogomph spiniger chaeta, dorsal fascicle, 10th chaetiger. **G.** Neuropodial heterogomph falciger chaeta, dorsal fascicle, 15th chaetiger.

estuary (39°23'26"S-73°11'44"W), 48 ind.; Mississipi, Valdivia (39°26'45"S-73°13'07"W), 18 ind.

#### Diagnosis

Length 39.8 mm (16.6-65.5), width 20 mm (9-32); number of chaetigers 84 (55-111). Prostomium slightly broader in the basal region, with a clear central line that runs through it longitudinally with a few small light-colored marks on the external edges. Two short antennae with distal ends of the same length as the palp or slightly longer. Two pairs of eyes forming an inverted trapezium. Two thick palps with rounded palpostyles, and well-distinguished from the prostomium. Four pairs of smooth tentacular cirri, the longest pair extending back to chaetiger 3 to 6. Thin jaws light brown, with 8-13 teeth on the internal border. The paragnaths (Fig. 2A-B) are arranged as follows: Area I = 0-6, frequently 2; Area II = 5-20 in a triangular arrangement; Area III = 21-55 irregularly arranged in a rectangular area; Area IV = 11-42 grouped in a semi-circle; Area V = 0-1 frequently only one; Area VI = 4-12 short bars arranged in a row; VII-VIII = 27-44 arranged in two rows. The size of the body segments subsequently decreases and ends in a pygidium with two smooth cirri ventrally. Notopodium with pointed lobes, with a shorter superior lobe than the inferior lobe in the posterior segments (Fig. 2C-D). The dorsal cirrus is basally inserted and has the same length as the superior notopodial ligula. Neuropodium with similar lobes; the ventral cirrus is three quarters the length of the neuropodial lobes. Notochaeta with homogomph spinigers only (Fig. 2E). Neurochaetae, dorsal fascicle: homogomph spinigers and heterogomph falcigers (Fig. 2F). Neurochaetae, ventral fascicle: heterogomph spinigers (Fig. 2G) and heterogomph falcigers.

Live specimens have a pale yellow color in the ventral region and brown in the dorsal region, with a red longitudinal line (dorsal blood vessel) that runs through the length of the body and with light brown lines similar to finger prints on both sides of each chaetiger.

#### Habitat

Estuaries with surface water salinity between 2.4 (mixo-oligohaline waters) and 34 (euhaline waters). *Perinereis gualpensis* is found in intertidal and subtidal zones of muddy sand and mudflats with abundant silt and clay.

#### Distribution

Endemic to Chile, in Concepción: Gualpén, Biobío River, Itata River, Lenga River (Jeldes, 1963; Bertrán et al., 2001; Diaz-Jaramillo et al., 2010); Valdivia: Lingue River, Queule River, Tornagaleones River (Bertrán, 1980 & 1989; Quijón & Jaramillo, 1993) and Aysén Fjords: Cuervo River (Cañete et al., 1999).

#### *Perinereis longidonta* Rozbaczylo & Castilla, 1973

*Perinereis longidonta* Rozbaczylo & Castilla, 1973: 221-225, Figs. 1b, 3 a-f.

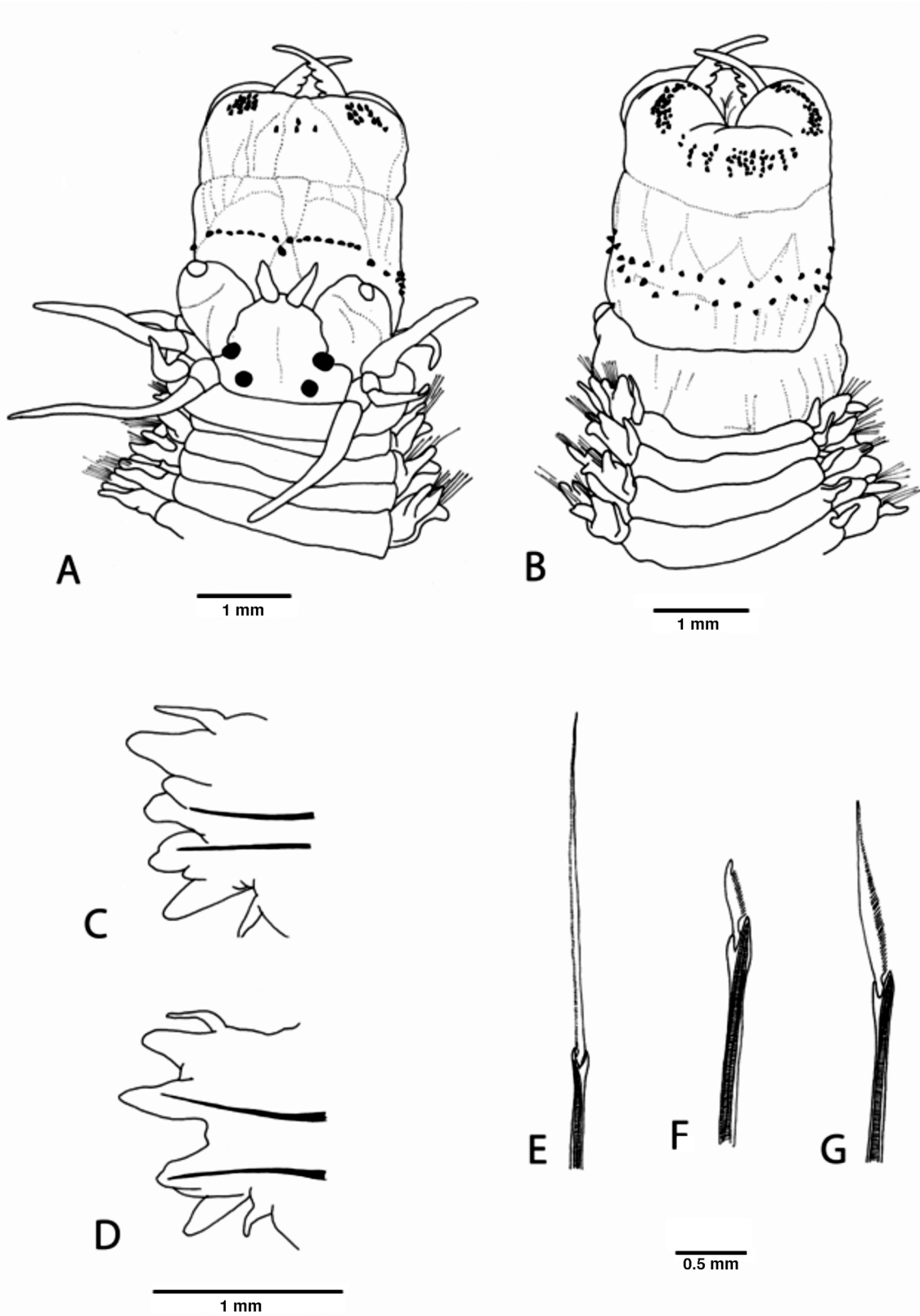
#### Material examined

Maicolpué, Osorno, 1 ind., (Colección de Flora y Fauna Profesor Patricio Sánchez Reyes, Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile); Pichicuyín beach, Valdivia (39°25'26"S-73°13'04"W), 5 ind.; Calfuco beach, Valdivia (39°47'34"S-73°23'32"W), 1 ind.

#### Diagnosis

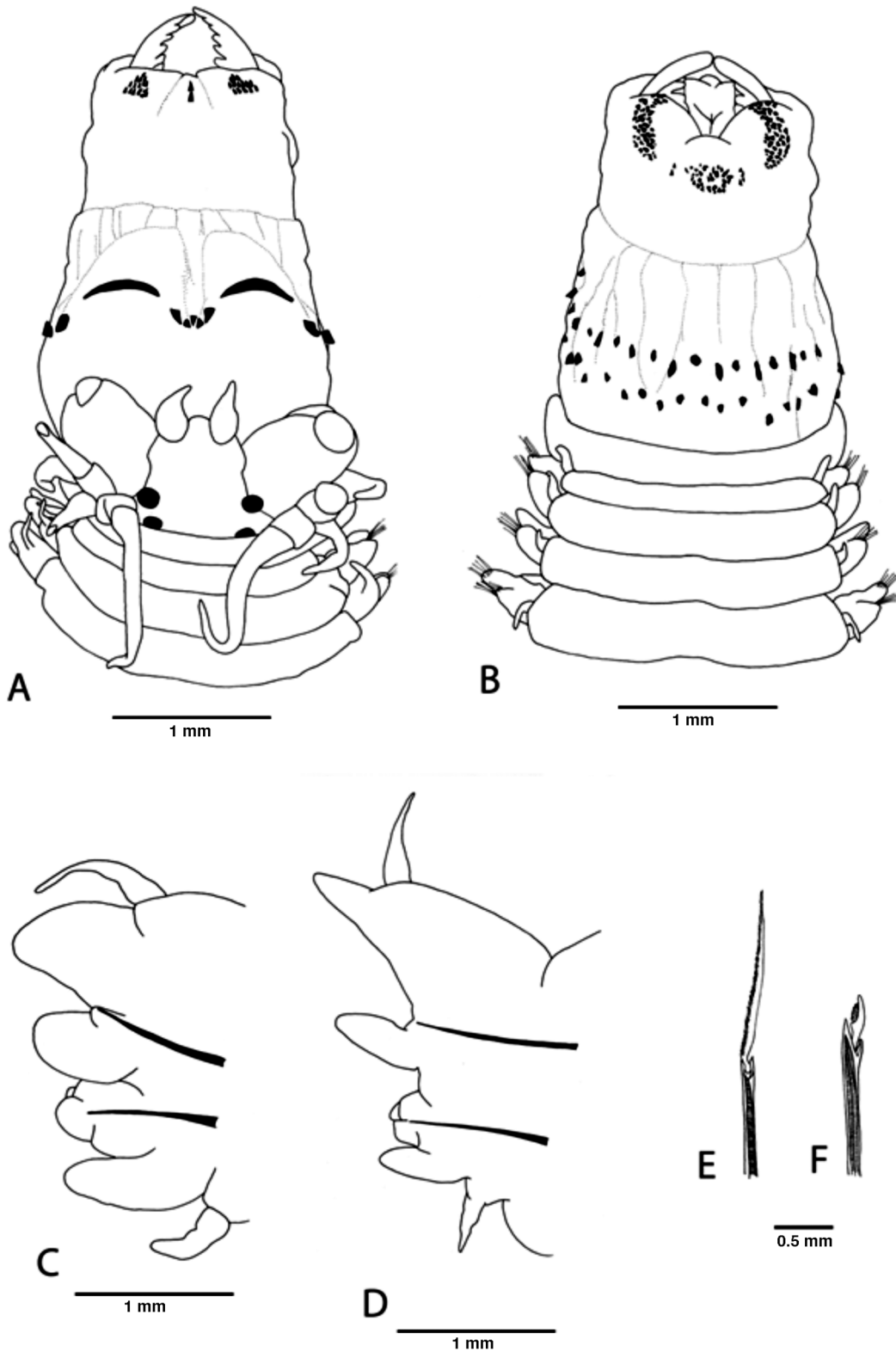
Length 68.4 mm (30-106), width excluding parapodia 3.8 mm (1-8); number of chaetigers 109 (86-119). Prostomium slightly longer than broader. Two short, conical antennae, shorter than the length of the palps. Four violet-colored eyes arranged in a trapezium shape, the anterior pair slightly larger than the posterior pair. Two palps with spherical palpostyles well-distinguished from the prostomium because of different pigmentation. Four pairs of short, smooth tentacular cirri, the longest pair extending back to chaetiger 2. Strong black jaws with 8 blunt teeth. The arrangement of paragnaths (Fig. 3A-B): Area I = 2-4 conical paragnaths of variable positions, sometimes in a vertical line, one after the other, arranged in two rows, one beside the other, or arranged in a cross-shape; Area II = 16-25 arranged in four rows forming a triangle; Area III = 22-55 small cones, forming an oval and lateral groups of 1-4 cones in a row on either side; Area IV = 58-82 cones; Area V = 3 cones forming a triangle; Area VI = 1 long, transverse, curved paragnath, at both sides of which there is always one conical paragnath with curved pointed-tips directed to Areas V and VIII, respectively; Area VII-VIII = 39-49 paragnaths arranged in two rows; each row presents paragnaths arranged alternately in two levels, the anterior row with cones and elongated paragnaths, the posterior row with alternately arranged cones. Anterior parapodia (Fig. 3C) with conical lobes of the same length; the superior notopodial lobe is slightly longer than the others, almost 3 times longer in the posterior segments (Fig. 3D); the other parapodial lobes are of a constant size throughout the body. Notochaetae: homogomph spinigers (Fig. 3E). Neurochaetae, dorsal fascicle; homogomph spinigers and heterogomph falcigers (Fig. 3F). Neurochaetae, ventral fascicle: heterogomph falcigers.

Live individuals are yellowish-brown to dark-brown with dorsal lines similar to finger prints. These prints are most pronounced towards the medial-posterior part of the body. This species is iridescent.



**Figure 2.** *Perinereis gualpensis* Jeldes, 1963. **A.** Anterior end, dorsal view. **B.** Anterior end, ventral view. **C.** Parapodium 10th. **D.** Parapodium 70th. **E.** Notopodial homogomph spiniger chaeta, 10th chaetiger. **F.** Neuropodial heterogomph falciger chaeta, dorsal fascicle, 10th chaetiger. **G.** Neuropodial heterogomph spiniger chaeta, ventral fascicle, 15th chaetiger.





**Figure 3.** *Perinereis longidonta* Rozbaczylo & Castilla, 1973. **A.** Anterior end, dorsal view. **B.** Anterior end, ventral view. **C.** Parapodium 9th. **D.** Parapodium 95th. **E.** Notopodial homogomph spiniger, 9th chaetiger. **F.** Neuropodial heterogomph falciger chaeta, dorsal fascicle, 10th chaetiger.

### Habitat

Intertidal marine zones associated with patches of *Peromytilus purpuratus* and *Pyura chilensis*.

### Distribution

Endemic to Chile, from El Tabo beach, Santiago to Maicolpué beach, Osorno (Rozbaczylo & Castilla, 1973).

### *Perinereis vallata* (Grube, 1857)

*Nereis vallata* Grube, 1857: 159-160; Ehlers, 1901a: 260; 1901b: 110-112 (partly).

*Perinereis nuntia* var. *vallata* Fauvel, 1932: 110-111; 1952: 296; 1953a: 215; 1953b: 8; Knox, 1951: 218-219; 1960: 122-124; Estcourt, 1967: 70; Rozbaczylo & Castilla, 1973: 225-226, figs. 4a-f.

*Perinereis vallata* Hartman, 1954; Wesenberg-Lund, 1962: 79-80; Hartmann-Schröder, 1962a: 412-417; 1962b: 108-110; 1965: 148,298; Bertrán, 1980:84-86, fig. 2.

*Perinereis nuntia vallata* Day, 1967: 334, figs. 14.12. p-5.

### Material examined

Cocholgué beach, Concepción (36°35'41"S-76°58'41"W), 40 ind.; Tumbes beach, Concepción (36°38'02"S-73°05'28"W), 2 ind.; Punta Lavapié (37°08'52"S-73°35'01"W), 1 ind.; Ancud (41°S-72°W), 4 ind. (Colección de Flora y Fauna Profesor Patricio Sánchez Reyes, Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile).

### Diagnosis

Length 56.3 mm (21-110), width excluding parapodia 2.2 mm (1-5), number of chaetigers 104 (59-143). Broaden prostomium in the basal region, with a lighter colored longitudinal middle line in some specimens. Two antennae shorter than the length of the palps. Two pairs of eyes, forming an inverted trapezium, the superior pair quite close to the inferior pair. Two palps, well-distinguished from the prostomium, with rounded palpostyles. Four pairs of tentacular cirri, slightly ringed, the longest pair extending back to chaetiger 7. The jaw is robust, of dark brown color. Large size specimens generally do not have teeth but have a cutting edge; individuals of smaller size have 7-8 teeth. Paragnath arrangement (Fig. 4A-B): Area I = 1-4 cones arranged in a row; Area II = 6-18 irregularly arranged; Area III = 11-26 arranged in 2 or 3 rows, with 1 or 2 smaller cones at both sides; Area IV = 22-42 arranged in short irregular rows; Area V = 1-3, commonly only one; Area VI = 8-16 short bars arranged in a transverse line; Area VII-VIII = 42-88 arranged in two rows, those closest to the peristomium are arranged irregularly. The body ends in a pygidium with two smooth cirri that originate ventrally.

The anterior parapodia present blunt ends. The notopodium of the anterior parapodia (Fig. 4C) presents a shorter superior lobe in comparison to the inferior lobe whereas in the posterior parapodia (Fig. 4D) the superior lobe is longer than the inferior lobe. Notochaetae: homogomph spinigers only (Fig. 4E). Neurochaetae, dorsal fascicle: homogomph spinigers (Fig. 4F) and heterohomph falcigers (Fig. 4G). Neurochaetae, ventral fascicle: heterogomph spinigers and heterogomph falcigers (Fig. 4H).

### Observations

Wilson & Glasby (1993) considered the presence-absence of paralleling bar-shaped paragnaths in Area IV of the proboscis, as an important character that located *P. vallata* into the group with bars present. However, this character has only been observed in few specimens used in this study. Then, this character was not considered in our analysis.

Live specimens are greenish-blue, somewhat iridescent, and darker in the first chaetigers and with lighter lines, similar to finger prints, which can be seen more clearly in posterior segments.

### Habitat

Middle and low tide zones, buried in thick sand, under large rocks and pebbles. In Cocholgué beach they are associated with the red alga *Gelidium*.

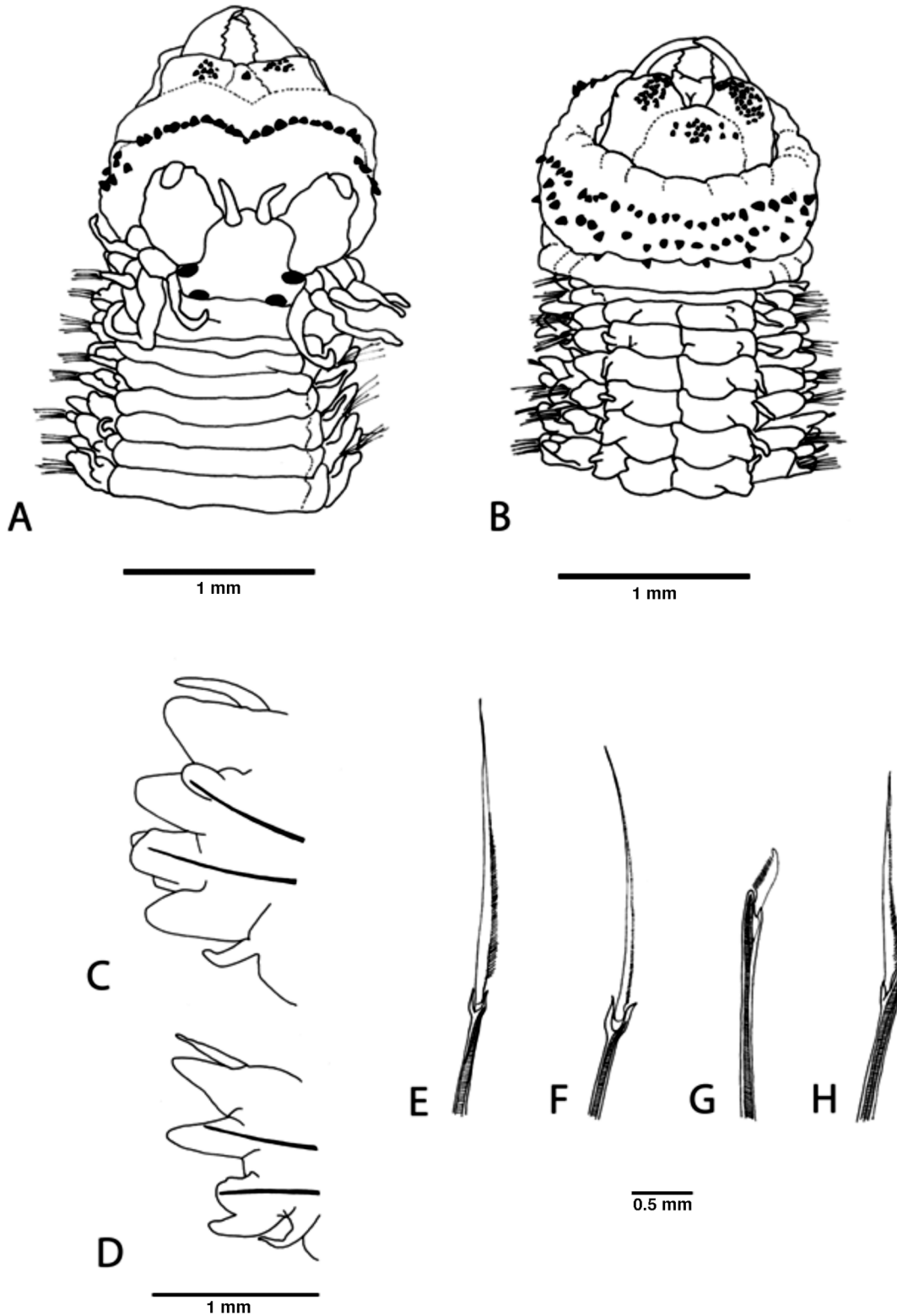
### Distribution

Southern Australia, from Albany to Botany Bay, including Macquarie Island; New Zealand; St. Paul Island; Chile, from Antofagasta to Aysén (Rozbaczylo & Castilla, 1973).

### Morphological phylogenetic analysis

A total of 89 characters was obtained from the nereidid literature (Bakken & Wilson, 2005; Santos et al., 2005; Bakken, 2007) and our descriptions. From these, only 26 were informative (Table 1). After a recoding into a binary matrix, we obtain 51 characters used in further analyses.

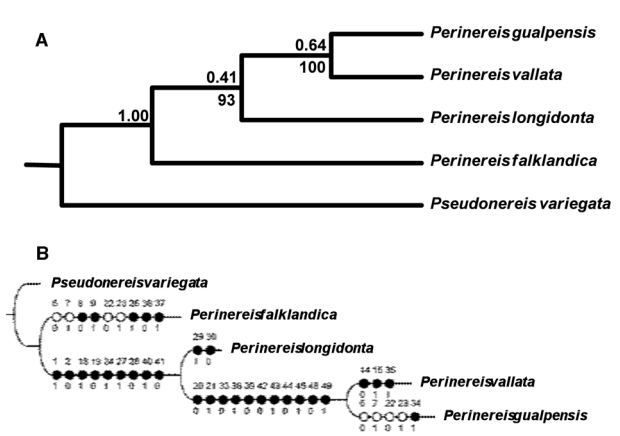
A similar topology was obtained with both MP and BMCMC analyses (Fig. 5A), nevertheless both differ in the branching support. MP resulted in one most parsimonious tree with 40 steps long, a Consistency Index of 0.75 and a Retention Index of 0.67. From the total of 51 characters, 32 were parsimony informative. In this tree, the four species of *Perinereis* are a monophyletic group (Fig. 5A). The clade formed by *P. gualpensis* and *P. vallata* is well-defined with a high bootstrap value and closer to *P. longidonta*, also grouped with a consistent bootstrap value (Fig. 5A). The Winclada analysis showed the synapomorphies and autapomorphies (Fig. 5B). Synapomorphies of *P. vallata* and *P. gualpensis* clade are: arrangement of paragnaths in Area III,



**Figure 4.** *Perinereis vallata* (Grube, 1857). **A.** Anterior end, dorsal view. **B.** Anterior end, ventral view. **C.** Parapodium 11th. **D.** Parapodium 70th. **E.** Notopodial homogomph spiniger chaeta. **F.** Neuropodial homogomph spiniger chaeta, dorsal fascicle, 11th chaetiger. **G.** Neuropodial heterogomph falciger chaeta, dorsal fascicle, 11th chaetiger. **H.** Neuropodial heterogomph spiniger chaeta, ventral fascicle, 15th chaetiger.

**Table 1.** Morphological matrix of the 26 character with informative value obtained for each species. ? = missing data; - = no applicable state.

|                               |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|                               | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |   |
|                               | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| <i>Perinereis longidonta</i>  | 1 | 2 | 2 | 1 | 3 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | - | - | 2 | 1 |
| <i>Perinereis falklandica</i> | 2 | 2 | ? | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 2 | - | 1 | 2 | 1 | - | - | 2 | 1 |
| <i>Perinereis vallata</i>     | 1 | 1 | 2 | 1 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | ? | 2 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 2 |
| <i>Perinereis gualpensis</i>  | 1 | 1 | ? | 2 | 3 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 2 |
| <i>Pseudonereis variegata</i> | 2 | 1 | ? | 1 | 3 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 2 | 1 | 2 | 1 | - | - | 2 | 2 |



**Figure 5.** A. Morphological phylogeny of *Perinereis* species used in this study. MP: 32 parsimony informative characters; tree length = 40 steps, CI = 0.75, RI = 0.67. BMCNC  $N = 82188$  trees. Numerals above and below branches indicate bootstrap support and posterior probability respectively. B. Winclada analysis performed on MP tree, showing apomorphies and homoplasies in black and white respectively. The numbers above the branches correspond to each character, and numbers below lines to character states.

forming an irregular patch; presence of more than one paragnath in Area VI, arranged in a transverse row of small bars; the form of the dorsal notopodial ligule in posterior chaetigers is similar both anterior and posterior chaetigers; prechaetal notopodial lobe absent and dorsal cirrus in basal position in the notopodial ligule of the posterior chaetigers. However, *P. gualpensis* show only one autapomorphic character, the paragnaths numbers in Area VI (6-10). This contrasting with the autapomorphies found in *P. vallata*, which are the paragnath number in Area VI (11-14) and the arrangement of paragnaths in Area II, forming an irregular patch. The autapomorphies found in *P. longidonta* are the presence of more than one paragnath in Area V. The synapomorphies that provided high node support values for the *P. longidonta*, *P. vallata* and *P. gualpensis* clade are: rounded palpostyles, paragnaths in Area III not arranged in rows, conical paragnaths in Area IV not grouped in a pattern and

conical dorsal notopodial ligule. *P. falklandica* presents as autapomorphies the paragnath arrangement in Area I, forming an irregular patch, presence of conical paragnaths and pointed bars in Area IV and paragnaths arrangement in Area VII-VIII forming a broad band. Homoplastic characters found between *P. falklandica* and *P. gualpensis* are: paragnaths in Area I not arranged in rows and the absence of conical paragnaths on both sides of Area III.

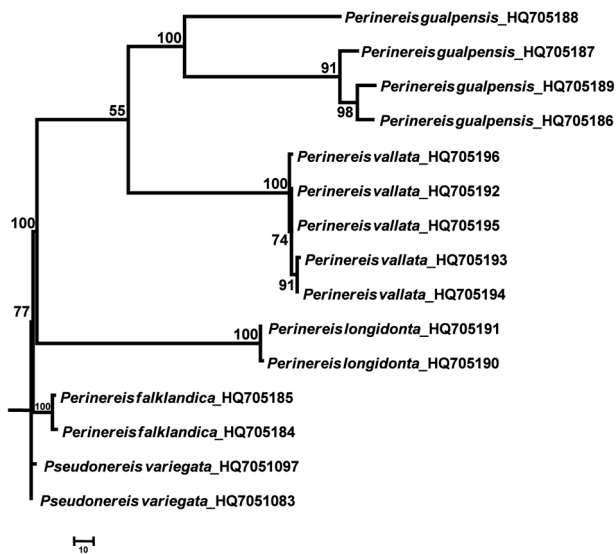
*Molecular phylogenetic analysis*

From the MP analysis, using a total of 704 characters, was obtained one tree with a length of 318 steps, a Consistency Index of 0.84 and a Retention Index of 0.94 (Fig. 6). The Chilean coast species of *Perinereis* comprise a monophyletic group, in which the four species of *Perinereis* (i.e. *P. falklandica*, *P. vallata*, *P. longidonta* and *P. gualpensis*) are well supported. *P. falklandica* is the sister group of the remaining species. The latter group, consists of *P. longidonta* as sister clade of (*P. gualpensis*, *P. vallata*) with a high bootstrap value.

By means of BMCNC, the model that provided the highest values of likelihood with least number of parameters was 2GTR+G. The majority-rule tree based on the CZ samples (Fig. 7) shows that the species of *Perinereis* are monophyletic. However this analysis shows differences with MP. *P. vallata*, *P. longidonta* and *P. gualpensis* comprise a monophyletic group, but *P. gualpensis* is the sister group of (*P. longidonta*, *P. vallata*). However, the posteriori probability obtained corroborates an uncertain relationship among these species. The only clade with a posteriori probability of 1.00 is the *P. vallata*, *P. longidonta* and *P. gualpensis*, while the relationship *P. vallata* and *P. longidonta* have a lower probability and are not shown as a well-supported clade (0.53).

**Discussion**

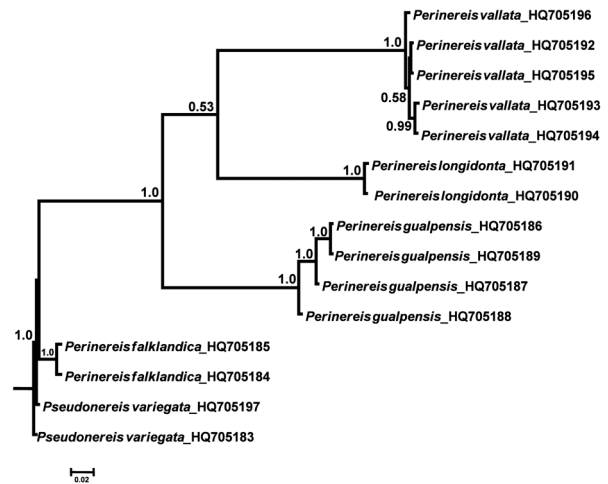
The taxonomic revision of four species of the genus *Perinereis* from the Chilean coast, Southeast Pacific, allows us to provide a detailed description of the most



**Figure 6.** MP tree from the COI mtDNA. Tree length = 318 steps, CI = 0.84, RI = 0.94. The numbers on nodes correspond to bootstrap values.

important traits at taxonomic and phylogenetic level. Among these, the type, number, and arrangement of paragnaths and especially the chaetal type and form of the notopodium. Given the great number of individuals used, was possible to cover a wider range of both, intra and inter-specific differences necessary for a reliable description of the species. The described taxa were similar in most morphological characters, with few apomorphies establishing differences between them. However, these few differences have proven to be useful to define each species (Fig. 5B). The use of morphological characters to evaluate relationships among Nereididae, is not problem-free. Given that the uniform anatomic structure in this family is difficult to discriminate between primary absence and secondary loss of characters, leading to high degrees of homoplasy (Bakken & Wilson, 2005; Santos et al., 2005). However, at more exclusive taxonomic level, the use of morphological characters in *P. gualpensis* and congeneric taxa from the Chilean coast has made possible to corroborate the specific validity for this species.

Phylogenies of polychaete families and species, using molecular characters, have increased in number. However, some of these works fail to support deepest basal nodes (Rousset et al., 2006). Explanations invoked to account this problem are a rapid radiation events for annelids, (i.e. the Cambrian explosion; Rousset et al., 2006), or the lack of phylogenetic signal because a limited samples of taxa and genes (Rouse & Pleijel, 2003). Our analyses based on molecular data have recovered the four species as a monophyletic group. However, the phylogenetic relationship



**Figure 7.** Consensus tree of the BMCMC approach based on the COI mtDNA. The values on nodes correspond to posterior probability.

among these species remains unclear, given that the phylogenetic topology depends on the method used. The MP analysis shows a closer relationship between *P. gualpensis* and *P. vallata*, whereas the BMCMC analysis shows that *P. gualpensis* is the sister group of (*P. vallata* + *P. longidonta*) (Figs 6 & 7). While these results could indicate both, wrong use or bad selection of data, alternative biological process could be invoked. For instance, if COI sequences present complex evolutionary processes, the MP algorithm could give erroneous results (Felsenstein, 2004). Alternatively, approaches such as BMCMC explicitly consider suitable substitution models for complex genes (Pagel & Meade, 2004). However, we obtain a posterior probability barely above 50% that support the monophyly of *P. vallata* and *P. longidonta* (Fig. 7). Under this context, the potential of COI gene as tool for *Perinereis* systematics, and particularly for the species studied here, can be challenged. Nevertheless, an invalidation of the molecular marker cannot be absolute. This based on two evidences: (i) Park & Kim (2007) showed a complete discrimination among four *Perinereis* species of Korea, using phylogenetic analysis based on COI; and (ii) our phylogenetic hypotheses show that each species correspond to a natural group. Therefore, the unstable topologies, at interior branches, obtained in our study could be evidence for complex speciation process. In this case, there are not simple bifurcation processes, or the rate of diversification of Chilean coast *Perinereis* is more accelerated than can be detected using this marker. Then, we propose the use of molecular markers that have a more accelerated rate of nucleotide substitution, to solve the inter-specific relationships among the Chilean coast *Perinereis*.

In spite of the complex relationships obtained, our results indicate that the estuarine species *P. gualpensis*

**Table 2.** List of the 26 binary and multistate morphological characters, which present a change of state. References: Bakken & Wilson (2005), Santos et al. (2005), Baken (2007).

|    |  |
|----|--|
| 1  | Palps, palpostyle shape: (1) spherical; (2) conical; (3) pointed.  |
| 2  | Peristomium, size: (1) similar to following segment; (2) longer than following segment;  |
| 3  | Epitoky: (1) absent; (2) present.  |
| 4  | Area I, paragnath arrangement: (1) in regular rows; (2) in irregular rows.   |
| 5  | Area I, paragnath distribution: (1) forming a regular cluster; (2) forming an irregular and compact cluster; (3) no forming a cluster.   |
| 6  | Area II, paragnath type: (1) conical; (2) pectinate.   |
| 7  | Area II, paragnath arrangement: (1) in regular rows; (2) in irregular rows.  |
| 8  | Area II, paragnath distribution: (1) forming a regular cluster; (2) forming an irregular and compact cluster; (3) no forming a cluster.  |
| 9  | Area III, paragnath type: (1) conical; (2) pectinate.  |
| 10 | Area III, paragnath arrangement: (1) in regular rows; (2) in irregular rows.   |
| 11 | Area III, paragnath distribution: (1) forming a regular cluster; (2) forming an irregular and compact cluster; (3) no forming a cluster. |
| 12 | Area III, conical paragnaths both sides: (1) absent; (2) present.  |
| 13 | Area IV, paragnaths type: (1) conical; (2) pointed bars; (3) conical and pointed bars  |
| 14 | Area IV, paragnath arrangement: (1) forming a regular cluster; (2) forming an irregular and compact cluster; (3) no forming a cluster.   |
| 15 | Area V, paragnath type: (1) conical; (2) pectinate.  |
| 16 | Area VI, paragnath shape: (1) smooth bar; (2) pointed tip bar; (3) shield-shaped bar.  |
| 17 | Area VI, bars number: (1) 1; (2) 6-11; (3) 12-14.  |
| 18 | Area VII-VIII, paragnath arrangement: (1) two rows (2) forming a broad band.   |
| 19 | Area VII-VIII, paragnath row arrangement: (1) row with similar sized cones; (2) row alternating cones and bars.                          |
| 20 | Dorsal notopodial ligule, shape: (1) rounded; (2) conical (3) enlarged; (4) branched.  |
| 21 | Dorsal notopodial ligule, feature on posterior chaetigers: (1) similar to anterior chaetigers; (2) elongated; (3) broader; (4) reduced.  |
| 22 | Prechaetal notopodial lobe: (1) absent; (2) present.   |
| 23 | Prechaetal notopodial lobe, size: (1) smaller than notopodial ligule; (2) as large as dorsal notopodial ligule.                          |
| 24 | Prechaetal notopodial lobe, range: (1) restricted to anterior chaetigers anteriores; (2) present throughout.                             |
| 25 | Dorsal cirrus, notopodial ligule attachment on posterior chaetigers: (1) basal; (2) mid-distal; (3) distal.                              |
| 26 | Neuropodial ventral fascicle, heterogomph spinigers: (1) absent; (2) present.  |

diversified from a marine ancestor. After gradual penetration from seawaters to estuaries, the populations were adapted to estuarine environment. This led to the populations of polychaetes to micro-geographical isolation and a subsequent reproductive isolation. It has been proposed that the colonization of estuaries leads to ecological changes and a rapid adaptive divergence in brackish populations from their marine ancestors, reinforced by multiple selective processes (Kelly et al., 2006). This model fits for estuarine Nereididae polychaetes. Some species have evolved variations in life cycle traits, mainly patterns of reproduction associated to the suppression of the epitoky, as well as the shift of the sex ratio towards the female sex and the reduction of the dispersal phase (e.g. *Perinereis rullieri* Prevedelli & Simonini, 2003). In *P. gualpensis* many aspects of these patterns have been observed (Guillermo Valenzuela, personal comment).

The analyses using molecular and morphological traits confirm the validity of *P. gualpensis*, *P. vallata*, *P. longidonta* and *P. falklandica*, and support the view of independent taxonomic units. Consequently, the taxonomic confusion about the possible sibling species *P. gualpensis* and *P. vallata* is completely resolved. However, morphological and genetics data are partially incongruent about the phylogenetic relationships of these four *Perinereis*

species. We concluded that different evolutionary process that affect the morphological and genetics data produce different answers. Also we propose the use of new molecular evidence arising from molecular markers with different nucleotide substitution rate to that used here.

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