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Unraveling the Genetic and Morphological Boundaries of the Kelps *Eisenia cokeri* and *E. gracilis* (Laminariales, Phaeophyceae) from Peru, and Their Phylogenetic Relationship with Eisenia from the Desventuradas Islands (Chile)

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Abstract

The kelp genus *Eisenia* Areschoug is represented by two species in the Southeast Pacific: *Eisenia cokeri*, distributed in Peru and Chile, and *E. gracilis*, endemic to Peru. However, the taxonomic distinction between these two species has been questioned. Additionally, it is uncertain whether *E. cokeri* is conspecific with the Northeast Pacific species *E. arborea*. To address these issues, we examine, for the first time, the morphology and the molecular phylogenetics of *E. cokeri* and *E. gracilis* across their geographic range. Sampling for morphological and molecular analyses was conducted at subtidal zones in five locations in Peru. Furthermore, a locality in Chile was sampled for molecular studies, where *E. cokeri* was documented. Peruvian *Eisenia* species exhibited notable differences in morphological characters, including size, holdfast diameter, stipe modifications (e.g., longitudinal division and pseudostipe formation), frond division, frond margins, and roughness, as evidenced by differences in thallus morphometric variables. Phylogenetic analyses using mitochondrial and chloroplast markers revealed three distinct genetic lineages and two endemic species: *E. cokeri* and *E. gracilis* from Peru, and a new *Eisenia* sp. from Chile. Moreover, we confirmed the distinctiveness of *E. cokeri* from *E. arborea* and provided updated information on the distribution of *E. cokeri* and *E. gracilis* in the Southeast Pacific. These findings are crucial for the management and conservation of these endemic, foundational species.

Keywords: endemic; genetic delimitation; genetic diversity; morphology; taxonomy

1. Introduction

Kelps are structurally complex brown seaweeds, mainly of the order Laminariales [1], widely distributed across more than 25% of coastal areas worldwide [1,2]. These algae can cover extensive benthic substrates and form highly productive and dynamic ecosystems called kelp beds or kelp forests that physically and biologically modify their surrounding environments [3,4]. Indeed, kelps are foundation species that facilitate the occurrence of a great variety of life forms, including

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mammals, fish, and invertebrates [6,7], and harbor many economically valuable species [5,6]. Despite the fact that these ecosystems provide a multitude of goods and services to society [7], valued at billions of dollars each year [10], basic aspects such as their taxonomy and phylogeny require greater attention.

Advances in molecular systematics have redefined the phylogenetic framework of the Laminariales [4], prompting significant revisions to their taxonomic circumscription. One major change was the reassignment of the genus *Eisenia* to the family Lessoniaceae, alongside *Ecklonia*, *Eckloniopsis*, *Egregia*, and *Lessonia* [4,8]. This assemblage is distinguished by the presence of sporophylls arising from the blades, a trait considered phylogenetically informative [9]. Multiple phylogenetic studies have further highlighted the close affinity between *Eisenia* and *Ecklonia* [4,9–11], two genera that exhibit striking morphological similarities, differing mainly in the mode of meristem division at the distal end of the stipe [12]. Based on mitochondrial and chloroplast markers, Rothman et al. proposed that *Eisenia arborea* Areschoug—the type species of the genus—should be regarded as conspecific with *Ecklonia*, raising the possibility that other *Eisenia* species might also require transfer [12]. However, subsequent multilocus analyses by Kawai et al. resolved *Ecklonia* and *Eisenia* as distinct, well-supported clades, thereby reaffirming *Eisenia* as a valid genus and retaining *E. arborea* as its generitype [13].

Despite significant progress in *Eisenia* phylogenetics, the taxonomic status of multiple species remains in need of formal revision. Previous molecular phylogenetic studies have primarily targeted conspicuous Northern Hemisphere representatives of the genus, including *E. arborea* and *E. bicyclis* [4,9–12], as well as *E. nipponica* [13]. In contrast, Southeast Pacific endemics such as *E. desmarestioides*, *E. galapagensis*, *E. cokeri*, and *E. gracilis* have remained unexamined at the molecular level since their original taxonomic descriptions.

Currently, *Eisenia* comprises seven species that are geographically restricted to the warm–temperate waters of the Pacific Ocean: *Eisenia arborea*, *Eisenia bicyclis* (Kjellman) Setchell, *Eisenia desmarestioides* Setchell & N. L. Gardner, *Eisenia galapagensis* W. R. Taylor, *Eisenia nipponica* H. Kawai, S. Akita, K. Hashimoto & T. Hanyuda, *Eisenia cokeri* M. Howe, and *Eisenia gracilis* E. Y. Dawson, Acleto & Foldvik [13–18]. Additionally, Buglass et al. reported a potential new species of *Eisenia* at depths exceeding 40 m near the Galapagos Islands, potentially increasing the number of recognized species within this genus to eight [19].

Subcanopy kelp *Eisenia* is found along the Peruvian coast, where it contributes to structuring subtidal beds and supports a diverse benthic community (see Carbajal et al. (2021) and Uribe et al. (2024) for further details) [6,20]. *Eisenia cokeri* has been recorded in several localities in Peru, between Máncora (5°S) and Paracas (15°S) [14,16,21], inhabiting hard substrates at depths of 0 and 12 m [6,22]. Additionally, it has been found in Chile, in the Desventuradas Islands and San Felix and San Ambrosio Islands (26°S) [15,17] from the intertidal zone to depths of up to 34 m [17]. In contrast, *E. gracilis* has been recorded in a single locality in Peru, Bahía San Juan (15° S), where it was dredged from sandy and shelly bottoms at 27 and 36 meters during the Allan Hancock Expedition in 1938 [16]. Based on the predictions of the potential occurrence of kelps in the deep waters of the eastern Pacific [23], *E. gracilis* may also be present in other locations along the central and southern Peruvian coast. However, this hypothesis has not yet been explored. Thus, *Eisenia* from Peru includes a low latitude shallow-water species (*E. cokeri*) and a mid-latitude deep species, potentially mesophotic (>30 m) (*E. gracilis*), influenced by the oceanographic conditions of the Humboldt Current Ecosystem. This system is characterized by permanent coastal upwelling that brings cold, low-oxygen, and nutrient-rich water to the surface, generating high coastal productivity [24,25]. Furthermore, the interannual phenomenon El Niño Southern Oscillation (ENSO) modifies these climatic conditions by reducing or canceling upwelling, and consequently increasing coastal temperatures [24,25].

Despite morphological differences exhibited by *E. cokeri* and *E. gracilis* [16], some doubts have emerged regarding the taxonomic identity of these species and other *Eisenia* species. In the past, the species status of *E. cokeri* and *E. gracilis* was questioned, with the suggestion that both could be conspecific [16,26]. Additionally, Howe highlighted the morphological resemblance of *E. cokeri* to *E.*

 doi:10.20944/preprints202512.0389.v1

arborea from North America [14] and suggested the conspecificity of these two taxa [16,17]. Also, the morphological differences between *E. cokeri* from Peru and those from the Desventuradas Islands [27], together with the large geographical distance separating these populations, raise doubts about the taxonomic unity of *E. cokeri*.

In this study, we conducted integrated molecular phylogenetic and morphological analyses of *Eisenia* species from the Peruvian coast to test the taxonomic distinctiveness of *E. cokeri* and *E. gracilis*. Furthermore, using multilocus molecular data, we evaluated the genetic cohesion of *E. cokeri* populations from Peru and Chile and assessed their divergence from the southern sea palm kelp, *E. arborea*.

2. Materials and Methods

2.1. Sampling Sites and Collection

Sampling was conducted between March and July 2018 across five localities spanning ~1,600 km of the Peruvian coastline. The sites were selected based on historical records [16,21] of *E. cokeri* and *E. gracilis*. This was complemented with one additional locality where the occurrence of *E. gracilis* was previously reported (Figure 1).

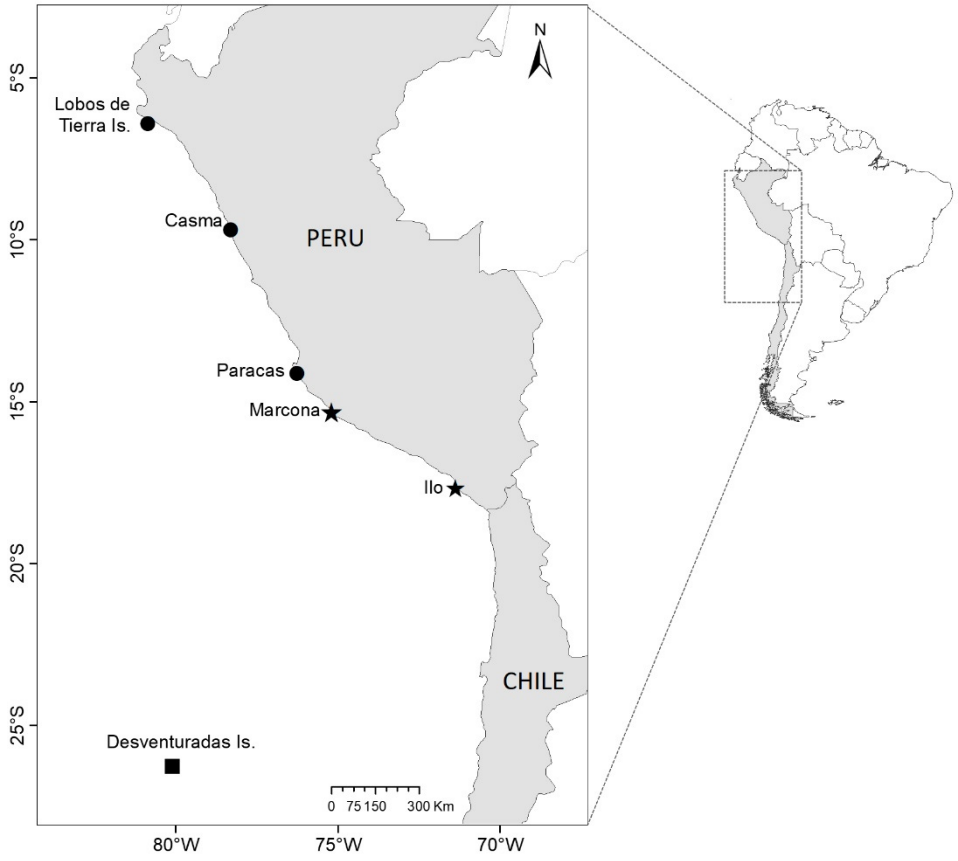

Figure 1. Map of sampling localities for *Eisenia cokeri* (circles) and *Eisenia gracilis* (stars) in Peru, and *Eisenia* specimens in Chile (square).

For *E. cokeri*, samples were collected from depths of 1 to 9 m at Lobos de Tierra Island, Casma, and Paracas, which together encompass 88% of the species geographic distribution in Peru (Figure 2A). For *E. gracilis*, we collected samples from Marcona, at a 22 m depth, and from Ilo (Figure 3A), at depths ranging from 10 to 15 m (Figure 1, Table 1).

 doi:10.20944/preprints202512.0389.v1

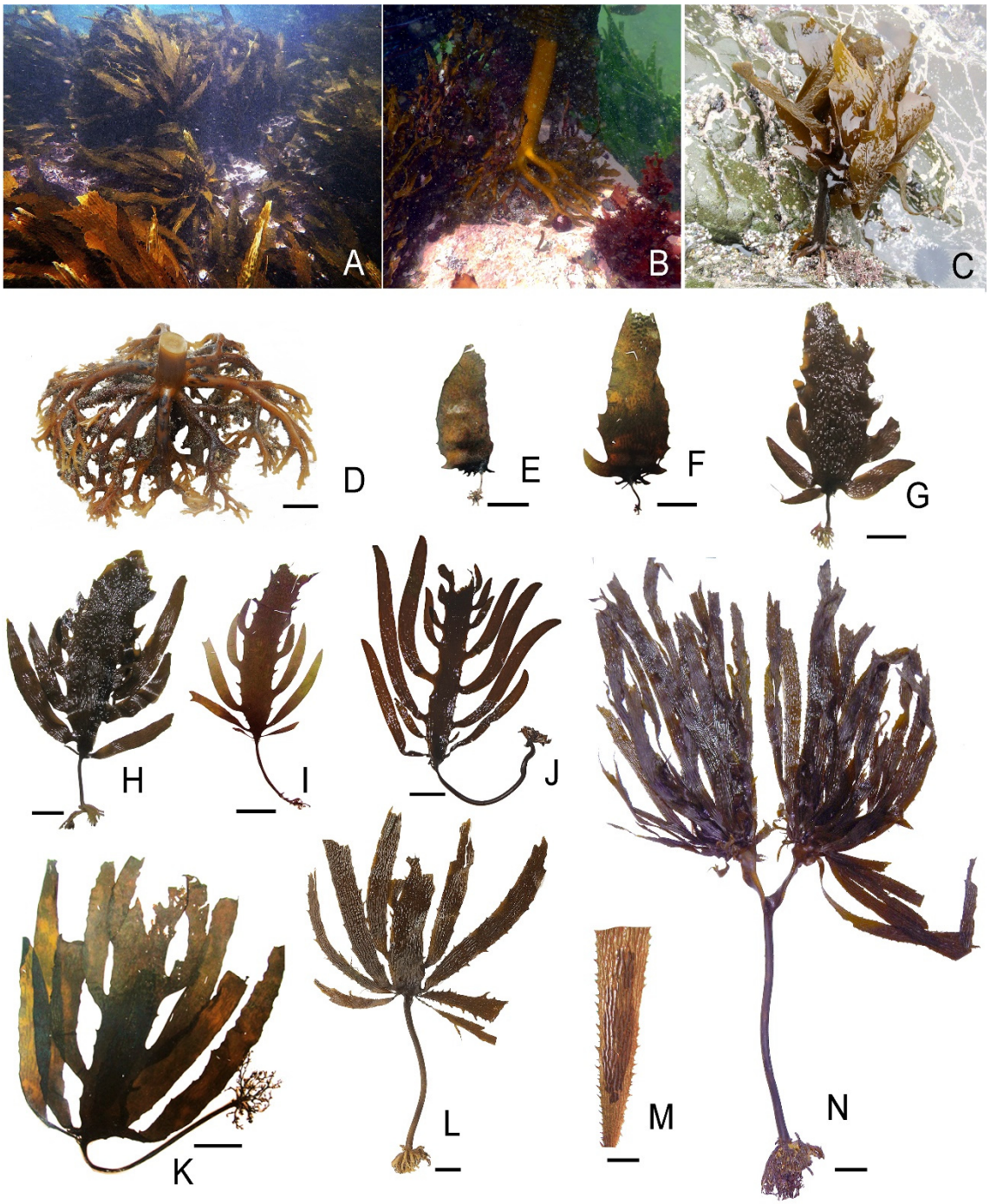

Figure 2. Habitat and morphology of *Eisenia cokeri* sporophytes from Peru. **(A)** Subtidal bed, Lobos de Tierra Island. **(B)** Holdfast attached to a rocky substrate, Lobos de Tierra Island. **(C)** Small adult specimen at a rocky intertidal shelf, showing a holdfast and a short stipe, Casma. **(D)** Detail of a solid stipe (arrow) and branched holdfast, Casma. **(E,F)** Entire juvenile fronds with scarce roughness, Casma. **(G)** and **(H)** Rough juvenile fronds bearing marginal pinnae of different sizes and smooth margins, Casma. **(I,J)** Juvenile specimens showing main and lanceolate secondary blades, Paracas and Huarmey (IMARPE 05-000343). **(K,L)** Juvenile fronds with a divided primary blade, a thickened base and serrated margins, Paracas. **(M)** Detail of a sporophyll, with serrated edges and sorus, Casma. **(N)** Specimen detached from subtidal populations, with a long stipe and blades grouped in two distal lateral branches, Rancherío. Scale bar = 5 cm. Photographs from P. Carbajal & N. Arakaki.

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Figure 3. Habitat and morphology of *Eisenia gracilis* sporophytes from Peru. **(A)** Subtidal bed, Ilo. **(B)** Holdfast attached to a rocky substrate, Ilo. **(C)** Detail of a branched holdfast with new stipes at the ends, Ilo. **(D)** Entire juvenile frond, Marcona. **(E)** Juvenile frond with short, spaced marginal pinnae, Marcona. **(F)** Juvenile specimen with alternately arranged and smooth-edged secondary blades, Marcona. **(G)** Adult uncommon specimen with short stipe, primary blade and serrated-edge secondary blades, Ilo. **(H)** Juvenile specimen with a divided primary blade, thickened base and long secondary blades, Marcona. **(I)** Blade detail with serrated edges with spaced teeth (right), and sorus (left), Ilo. **(J)** Adult specimen appearing fork-like, showing a long stipe, divided primary blade, and long lateral fronds, Ilo. Photographs from P. Carbajal & N. Arakaki.

In each locality, we randomly collected plants and thallus fragments via semiautonomous diving. A minimum of 30 adult *Eisenia* sporophytes were sampled for morphometric measurements, and 30 epibiont-free fronds, from plants separated by at least 1 meter, were obtained and immediately preserved in silica gel for phylogenetic analysis. Also, a total of 14 thallus samples of *E. cokeri* were

 doi:10.20944/preprints202512.0389.v1

collected from the San Ambrosio Islands, Chile (hereafter referred to as *Eisenia* from the Desventuradas Islands or Chilean *Eisenia*) for molecular analysis.

Table 1. Collection information and GenBank accession numbers for the specimens of *Eisenia* from Peru and Desventuradas Islands, Chile, included in the molecular analysis. *Eisenia* from Desventuradas Islands is considered as a species not a priori determined.

Species (Area)	Locality	Accession Number				
		ATP8S	COI	WI	RS	*rbc*L
E. cokeri (Peru)	Lobos de Tierra Island, Lambayeque, coll. P. Carbajal & Iván Gómez, 06°25'30.4" S, 80°51'58.9" W	OR361924	OR361839	OR362016	OR362119	OR361884
		OR361925	OR361840	OR362017	OR362120	OR361885
		OR361926	OR361841	OR362018	OR362121	OR361886
		OR361927	OR361842	OR362019	OR362122	OR361887
		OR361928	OR361843	OR362020	OR362123	OR361888
		OR361929	OR361844	OR362021	OR362124	OR361889
		OR361930		OR362022		
		OR361931		OR362023		
		OR361932		OR362024		
		OR361933		OR362025		
		OR361934		OR362026		
		OR361935		OR362027		
		OR361936		OR362028		
		OR361937		OR362029		
		OR361938		OR362030		
E. cokeri (Peru)	Casma, Ancash, coll. D. Hinostroza, 09°42'22.8" S, 78°17'49.8" W	OR361939	OR361845	OR362031	OR362125	OR361890
		OR361940	OR361846	OR362032	OR362126	OR361891
		OR361941	OR361847	OR362033	OR362127	OR361892
		OR361942	OR361848	OR362034	OR362128	OR361893
		OR361943	OR361849	OR362035	OR362129	OR361894
		OR361944		OR362036		
		OR361945		OR362037		
		OR361946		OR362038		
		OR361947		OR362039		
		OR361948		OR362040		
		OR361949		OR362041		
		OR361950		OR362042		
		OR361951		OR362043		
		OR361952		OR362044		
		OR361953				
E. cokeri (Peru)	El Ancla, Paracas, Ica, coll. P. Carbajal, 14°09'10.5" S, 76°14'52.9" W	OR361954	OR361850	OR362045	OR362130	OR361895
		OR361955	OR361851	OR362046	OR362131	OR361896
		OR361956	OR361852	OR362047	OR362132	OR361897
		OR361957	OR361853	OR362048	OR362133	OR361898
		OR361958	OR361854	OR362049	OR362134	OR361899
		OR361959		OR362050		
		OR361960		OR362051		
		OR361961		OR362052		
		OR361962		OR362053		
		OR361963		OR362054		
		OR361964		OR362055		
		OR361965		OR362056		
		OR361966		OR362057		
		OR361967		OR362058		
		OR361968		OR362059		
E. cokeri (Peru)	Rancherío, Laguna Grande, Ica, coll. P. Carbajal, 14°15'10.5" S, 76°25'52.9" W	OR361969	OR361855	OR362060	OR362135	OR361900
		OR361970	OR361856	OR362061	OR362136	OR361901
		OR361971	OR361857	OR362062	OR362137	OR361902
		OR361972	OR361858	OR362063	OR362138	OR361903
		OR361973	OR361859	OR362064	OR362139	OR361904
		OR361974	OR361860	OR362065		
		OR361975	OR361861	OR362066		
			OR361862	OR362067		

 doi:10.20944/preprints202512.0389.v1

			OR361863	OR362068		
			OR361864	OR362069		
				OR362070		
				OR362071		
				OR362072		
				OR362073		
				OR362074		
Eisenia (Desventuradas Is.)	San Ambrosio Island, Desventuradas Island, coll. P. Manríquez Ángulo, 26°20'12.8" S, 79°53'31.4" W	OR361976	OR361865	OR362075	OR362140	OR361905
		OR361977	OR361866	OR362076	OR362141	OR361906
		OR361978	OR361867	OR362077	OR362142	OR361907
		OR361979	OR361868	OR362078	OR362143	OR361908
		OR361980	OR361869	OR362079	OR362144	OR361909
		OR361981	OR361870	OR362080	OR362145	OR361910
		OR361982		OR362081		
		OR361983		OR362082		
		OR361984		OR362083		
		OR361985		OR362084		
		OR361986		OR362085		
		OR361987		OR362086		
		OR361988		OR362087		
		OR361989		OR362088		
E. gracilis (Peru)	Tres Hermanas, Marcona, Ica, coll. P. Carbajal, 15°26'29.1" S, 75°04'32.5" W	OR361990	OR361871	OR362089	OR362146	OR361911
		OR361991	OR361872	OR362090	OR362147	OR361912
		OR361992	OR361873	OR362091	OR362148	OR361913
		OR361993	OR361874	OR362092	OR362149	OR361914
		OR361994	OR361875	OR362093	OR362150	OR361915
		OR361995	OR361876	OR362094	OR362151	OR361916
		OR361996	OR361877	OR362095	OR362152	OR361917
		OR361997	OR361878	OR362096	OR362153	OR361918
		OR361998		OR362097		
		OR361999		OR362098		
		OR362000		OR362099		
		OR362001		OR362100		
				OR362101		
				OR362102		
				OR362103		
E. gracilis (Peru)	Leonas, Ilo, Moquegua, coll. P. Carbajal, 17°40'39.3" S, 71°22'15.9" W	OR362002	OR361879	OR362104	OR362154	OR361919
		OR362003	OR361880	OR362105	OR362155	OR361920
		OR362004	OR361881	OR362106	OR362156	OR361921
		OR362005	OR361882	OR362107	OR362157	OR361922
		OR362006	OR361883	OR362108	OR362158	OR361923
		OR362007		OR362109		
		OR362008		OR362110		
		OR362009		OR362111		
		OR362010		OR362112		
		OR362011		OR362113		
		OR362012		OR362114		
		OR362013		OR362115		
		OR362014		OR362116		
		OR362015		OR362117		
				OR362118		

Additionally, qualitative observations of the *Eisenia* habitat (bathymetric range and bottom type) were made. The depth range was estimated from perpendicular dives from the intertidal zone to the depth at which the *Eisenia* record was null for at least three contiguous meters, or up to a maximum depth of 30 m, due to diving safety restrictions. Sampling at the Desventuradas Islands was not possible due to this site's inaccessibility.

Sporophyte habit descriptions were carried out using fresh and preserved specimens (i.e., herbarium exsiccate) deposited in the Instituto del Mar del Perú Herbarium, collected between 2008 and 2019 across Peruvian coastal localities. Morphological features were complemented by field observations and photographs of *E. cokeri* from Lobos de Tierra Island (6°S) and Rancherío (14°S),

 doi:10.20944/preprints202512.0389.v1

between 2018 and 2019. Additionally, unpublished data from maximum holdfast diameter, stipe length, total length, and total fresh weight of *E. cokeri* from Casma (2009 and 2015) and total length of *E. gracilis* from Marcona (2015) were included (Supplementary Table S1).

We compared our information with the original descriptions of *E. cokeri* [14] and *E. gracilis* [16]. Due to logistical constraints associated with the remoteness of the Desventuradas Islands, no morphological observations of *Eisenia* could be made; therefore, descriptions were based on previous studies.

We randomly collected a minimum of 30 adult *Eisenia* sporophytes per locality to measure in situ the following morphometric variables: maximum holdfast diameter (MHD), holdfast height (HH), stipe length (SL), stipe diameter (SDI), and total length (TL). In addition, biomass was quantified as the total fresh weight (TFW). Descriptive statistics (mean, standard deviation, minimum, and maximum) were computed for each species. Differences in morphometric variables and biomass between *E. cokeri* and *E. gracilis* were evaluated using the pairwise Mann–Whitney U test. This non-parametric test was used due to non-normal and heteroscedastic distributions of most variables, as evidenced by the Shapiro–Wilk and Levene tests, respectively. The threshold for statistical significance was set at a p-value of 0.05. To graphically explore morphometric differences between the two *Eisenia* species, Principal Component Analysis (PCA) was applied after square root transformation and data normalization. A multivariate non-parametric permutational ANOVA (PERMANOVA) [31] was performed to assess significant differences in morphometric variables between the two kelps. For this purpose, similarity matrices based on Euclidean distances of the untransformed data were used, with 9999 random permutations generated using the unrestricted permutation method applied to the raw data. Statistical analyses were performed using R statistical software (version 4.4.1, Core Team 2024) and the PRIMER v7 program [28].

2.2. Phylogenetic Analyses

DNA extraction was performed from the pulverized algae using a modified protocol of the Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific). Five genetic markers from two cellular compartments were evaluated: (i) three mitochondrial markers: *atp*8-trnS or ATP8S (intergenic spacer between the *atp8* and *trnS* genes), trnW-trnI or WI (intergenic spacer between the *trnW* and *trnI* genes), and COI (cytochrome oxidase C subunit I gene), and (ii) two chloroplast markers: *rbc*L (RuBisCo or ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) and the *rbc*L spacer or RS (spacer between the *rbc*L and *rbc*S genes) (Supplementary Table S2). For the ATP8S and WI markers, we used the primers described by Engel et al. [33] and the PCR mixture and program used by Voisin et al. [29]. For COI, we used the GazF1 and GazR2 primers designed by Lane et al. [35], and the PCR mixture and program described by Macaya & Zuccarello [30]. For *rbc*L, we used the KL2 and KL8 primers [4] and the PCR recipe and program described by Rothman et al. [12]. The RS1 and RS2 primers [10] were used for amplification of RS (Supplementary Table S2). PCR products were verified by 1% agarose gel electrophoresis, purified, and sequenced using an ABI 3730xl automatic DNA sequencer from Macrogen Inc. (Korea). For each marker, the obtained sequences were edited using Chromas v.2.5.1 [31] and multiple sequence alignments were performed with the CLUSTALW function of BioEdit 7.2.5 [32].

For each marker, the sequence alignment was performed using *Eisenia*, *Ecklonia*, and *Lessonia* sequences available in GenBank. To infer phylogenetic relationships between *Eisenia* and *Ecklonia* species, the trees were rooted with Laminariales species (Supplementary Table S3). The construction of the trees was based on the options and parameters described by Tellier et al. [33]. The JModelTest2 program [34] was employed to determine the optimal model of sequence evolution for each marker and the consensus (all markers concatenated), using the Akaike Information Criterion (AIC). The GTR+I+G model was identified as the best fit for both markers. Phylogenetic relationships were inferred using Maximum Likelihood (ML) in the IQ-TREE program [35] with 1,000 bootstrap replications, and Bayesian Inference (BI) implemented in MrBayes 3.2.1 [36] with 10 million generations. Trees were sampled every 1,000 generations during the run, and the first 30% were

 doi:10.20944/preprints202512.0389.v1

discarded as burn-in. The resulting phylogenetic trees were visualized and edited using Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Sequences of the Laminariales order were used for the outgroup (*Macrocystis pyrifera*) selection, as performed by Rothman et al. [12] and Kawai et al. [13].

For each marker, molecular diversity indices were estimated at the species and locality level: the number of haplotypes, private haplotypes, and polymorphic sites, the haplotype diversity (H), and the nucleotide diversity (π) in the program DnaSP 5.10.01 [37]. We calculated differences between groups as mean pairwise p-distances for intraspecific and interspecific comparisons using MEGA 7.0.14, excluding positions with indel polymorphisms. Haplotype networks were constructed for each marker using the Median Joining algorithm of NETWORK v.5.0 [38]. The reticulations and loops were solved according to Crandall & Templeton [39].

2.3. DNA-Based Species Delimitation Method with GMYC

Prior to delimitation analyses, sequences were collapsed into unique haplotypes. For the GMYC analysis, an ultrametric tree was constructed in BEAST v.1.8 [40] for each marker (Supplementary Table S3) using the appropriate model selected by JModelTest [40] and assuming an uncorrelated lognormal relaxed molecular clock under the constant-size coalescent model. Fifty million generations were implanted for independent runs, sampling every 1000 trees. Runs were inspected for convergence using Tracer v.1.5, and trees were summarized from the MCMC analyses after discarding the first 25% of trees generated. GMYC analyses were run using a single threshold following Fujisawa & Barraclough [41] using the SPLITS package in R [42].

3. Results

3.1. Morphological Observations

3.1.1. *Eisenia cokeri*

Additional specimens examined: Lambayeque, Lobos de Tierra Island, subtidal, coll. A. Indacochea, IMARPE 05-000263, 06° 24′ 25.8'' S, 80° 51′ 50.5″ W, 08.x.2008. Ancash, Huarmey, Islote Patillos, Culebras, subtidal, coll. A. Gamarra, IMARPE 05-000343, 09° 53′ 44.0″ S, 78° 14′ 14.2″ W, 03. x.2015. Ica, Paracas, Rancherío, drift, coll. J. Zavala, IMARPE 05-000279, 16.vii.2008.

Description: Sporophyte thalli up to 1.8 m high and up to 1.2 kg in weight. Holdfast of densely intertwined haptera (Figure 2A–B, D), with a diameter of up to 34 cm and a height of 16 cm. The sporophyte exhibited a solid stipe, measuring 1.6 cm in diameter and reaching lengths of up to 50 cm, terete at the base and transitioning to a flattened and compressed form in the upper portion (Figure 2C). Juvenile fronds are lanceolate–oblong, initially smooth and then longitudinally roughened (rugose) (Figure 2E), reaching widths of up to 11 cm and exhibiting falcate lanceolate lateral bladelets with whole margins (Figure 2F–H). The main blade, thickened and elongated, included lanceolate, entire-edged bladelets that transformed into secondary blades, which reduced in size toward the apex (Figure 2I–J). The main blade began to erode at the apex while its base thickened to form entire lanceolate blades (Figure 2K–L), with serrated margins and spines of different sizes (Figure 2M). The mature main blade splits into two false stipes or compressed, flattened branches, which twist, culminating in a broad, compressed base that supports several simple, long blades reaching up to 1.6 m in length, which are occasionally divided. Mature blades are thick serrated, and exhibit longitudinal rugosity (Figure 2N). Sporangia are clustered in sori that form confluent, subcontinuous bands.

Remarks: Previous measurements of *E. cokeri* conducted in 2009 and 2015 in Casma attained maximum values exceeding those reported here for total thallus height (2.7 m, measured specimens = 103), total fresh weight (2.5 kg, measured specimens = 37), maximum holdfast diameter (28 cm, measured specimens = 51), and stipe length (92 cm, n=16).

Habitat: *Eisenia cokeri* beds (Figure 2A) were observed in Peru in the lower intertidal zone to depths of approximately 10 m, in locations protected from wave exposure (Lobos de Tierra Island

 doi:10.20944/preprints202512.0389.v1

and Paracas) as well as in places with moderate level of wave exposure (Casma). Sporophytes were mainly observed on platforms and irregular rocky blocks, pebbles, and small rocks, surrounded by coarse sand or sandy-shingle bottoms. In all assessed locations, *E. cokeri* formed monospecific kelp beds.

3.1.2. *Eisenia gracilis*

Additional specimens examined: Ica, Carrizales, subtidal, coll. A. Gamarra, IMARPE 05-001683, 15° 26′ 15.7″ S, 75° 04′ 46.0″ W, 29.xi.2015. Ica, San Juan de Marcona, subtidal, coll. J. Zavala, IMARPE 05-000279, 16.vii.2008.

Description: Sporophytes reach a height of 1.1 m and a weight of 0.09 kg. Holdfasts exhibiting sparsely branched haptera and rhizomatous development from which new stipes (stolon-like) emerged (Figure 3A–C). The stipe is subcylindrical at the base, measuring up to 0.8 cm in diameter, then becomes flattened, attaining a length of up to 52 cm. Juvenile fronds are initially lanceolate and smooth (Figure 3D), thereafter developing marginal bladelets that are spread apart, some falcate, placed alternately, with smooth margins (Figure 3E–F). Some mature individuals have primary blades and secondary blades with strongly serrated margins (Figure 3G). Most individuals exhibit a compressed primary blade base, generating 3–6 additional linear, lanceolate, serrate blades of similar width (Figure 3H). Sporangia aggregated in sori, forming confluent, subcontinuous bands (Figure 3I). Fronds featuring thick, serrated mature blades concentrated at the distal region of the thallus, exhibiting an elongated stipe reaching up to 52 cm (Figure 3J).

Remarks: Measurements of *E. gracilis* obtained in Marcona in 2011 and 2015 exhibited greater total thallus height (1.5 m, measured specimens = 38) and total fresh weight (0.5 kg, measured specimens = 21) than those recorded in this study.

Habitat: *Eisenia gracilis* (Figure 3A) was located at depths ranging from 10 to 30 m (Marcona: 20–30 m, Ilo: 10–15 m), in regions with moderate to high wave exposure, on both rocky surfaces and biogenic substrates. In Marcona, this species was observed to grow on mytilid *Aulacomya ater* communities, while in Ilo, it was found on small, scattered blocks, pebbles, and rocks located between sandy shell substrates and boulders. At both sites, *E. gracilis* was observed producing monospecific or mixed aggregations, cohabiting with *Lessonia trabeculata*. In the latter case, *E. gracilis* grew on the flanks of the rocky blocks already colonized by *L. trabeculata* (Ilo). The discovery of *E. gracilis* in Ilo extends the known distribution area of this species.

3.1.2. Eisenia from Desventuradas Islands

Remarks: Thalli attained a maximum length of 1 m [17], characterized by a holdfast including intertwined haptera and a robust, compressed, and flattened stipe. The blades were oblong at the base and linear–lanceolate, measuring 1.3 to 2.1 cm wide and 20 to 30 cm long. All blades showed pronounced longitudinal grooves and submarginal toothed margins [15,27]. The blades of *Eisenia* from Desventuradas exhibit an intermediate morphology between the Peruvian *E. cokeri* and *E. gracilis*, characterized by longitudinal grooves and a narrow linear–lanceolate shape, respectively [27].

Habitat: *Eisenia* from the Desventuradas Islands (San Ambrosio Island) has been recorded at depths ranging from the intertidal zone to 34 m, establishing dense populations on rocky bottoms with high wave exposure [27,43].

3.2. Morphometric Analysis

Descriptive statistics of the morphometric variables measured in situ in 101 specimens of *E. cokeri* and in 67 of *E. gracilis* are presented in Table 2. Morphometric descriptors, including maximum holdfast diameter, holdfast height, stipe diameter, total length, and total fresh weight, were significantly higher in *E. cokeri* than in *E. gracilis* (Mann–Whitney, $p<0.05$; see Supplementary Table S4). Four variables (maximum holdfast diameter, holdfast height, stipe diameter, and total fresh

 doi:10.20944/preprints202512.0389.v1

weight) of *E. cokeri* exceeded twice the metrics of *E. gracilis*, yet the most notable difference among both species was found in their biomass (Table 2). The morphological differentiation between the two *Eisenia* species was further evidenced by the PCA plot (Figure 4), which showed that each species formed a well-defined group. The first and second principal components adequately captured the variation in thallus morphology, explaining 86% (PC1: 69%, PC2: 17%) of the variance across all observations. *E. gracilis* plants were associated with lower values of morphometric variables and also showed less morphological variation, forming a more cohesive cluster of points compared to the more dispersed observations of *E. cokeri* (Figure 4). The morphological distinctiveness of both *Eisenia* was significantly supported by PERMANOVA analysis (Pseudo-F = 169.04, P < 0.001).

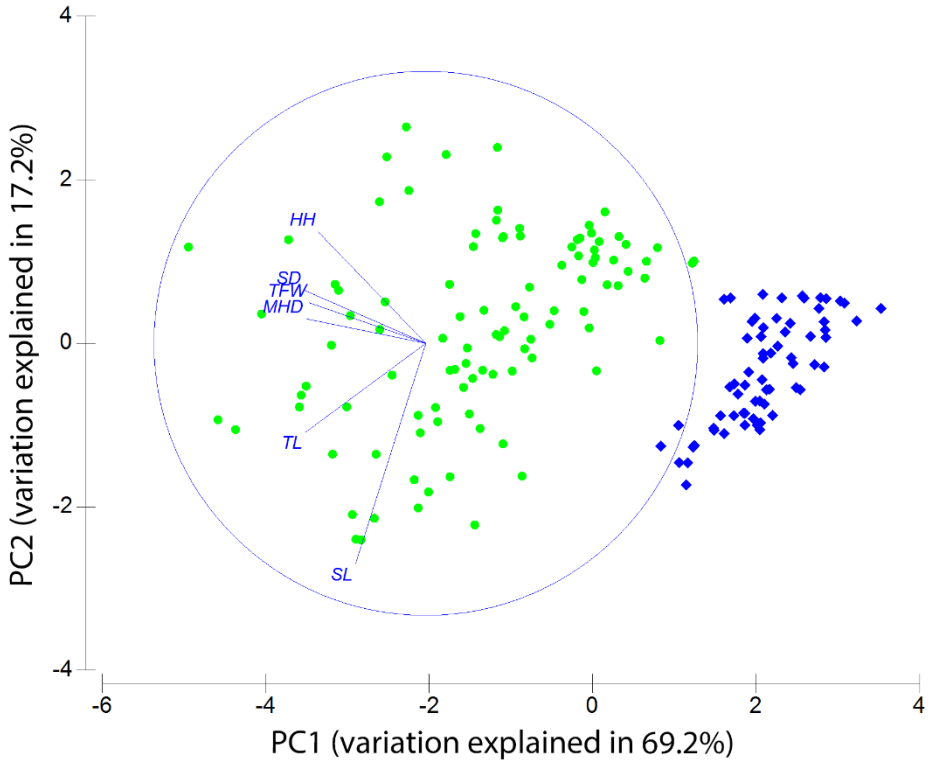

Figure 4. Principal Component Analysis of morphological variables and biomass of *Eisenia cokeri* (green) and *Eisenia gracilis* (blue). MHD: maximum holdfast diameter; HH: holdfast height; SL: stipe length; SDI: stipe diameter; TL: total length; TFW: total fresh weight.

Table 2. Morphometric variables and biomass of *Eisenia cokeri* and *Eisenia gracilis* collected in Peru during 2018 and 2019.

Variable		*E. cokeri*	*E. gracilis*
Maximum holdfast diameter (cm)	Mean	18,8	7,1
	DE	4,0	2,8
	Min	11,0	2,0
	Max	33,5	13,0
Holdfast height (cm)	Mean	7,4	2,7
	DE	2,7	1,0
	Min	3,0	1,0
	Max	16,0	6,0
Stipe length (cm)	Mean	32,5	24,8
	DE	20,9	13,2
	Min	4,4	6,0
	Max	84,5	52,0
Stipe diameter (cm)	Mean	1,4	0,5
	DE	0,3	0,1

 doi:10.20944/preprints202512.0389.v1

	Min	0,9	0,3
	Max	2,3	0,8
Total length (cm)	Mean	132,4	70,4
	DE	41,6	21,9
	Min	1,3	24,0
	Max	220,0	114,0
Total fresh weight (g)	Mean	815	25
	DE	536	16
	Min	80	5
	Max	2780	90

3.3. Delimitation of Species by GMYC Analyses

GMYC analyses (Table 3) across the five genetic markers (ATP8S, WI, COI, RS, and *rbc*L) consistently supported the presence of three *Eisenia* species in the Southeastern Pacific (SEP), including the Peruvian coast. Although the number of putative GMYC clusters varied among markers—ranging from 9 to 14 for COI, RS, and *rbc*L, and 10 for ATP8S—this variation mainly reflects intraspecific structure rather than additional species. WI showed no significant support for the GMYC model, indicating lower resolution for lineage discrimination. Overall, despite differences in clustering depth, all markers converge on the same biological conclusion: three well-delimited *Eisenia* species occur in Peru and the broader SEP region.

Table 3. Species delimited using different gene regions implemented in GMYC using a single threshold.

	Laminariales				
	ATP8S	**WI**	**COI**	**RS**	***rbc*L**
Likelihood of null model	538	815	569	1651	667
Maximum likelihood of GMYC	543	815	597	1678	668
Likelihood ratio	11**	0.14 n.s	6***	574***	15***
Number of ML clusters (95% CI)	10 (8–12)	13 (6–21)	9 (8–10)	12 (11–13)	14 (12-16)
Number of *Eisenia* species in SEP	3	3	3	3	3

3.4. Phylogenetic Analysis

A total of 277 sequences were generated (Table 4), comprising five molecular markers. Sequences for ATP8S and WI were trimmed to 162 and 174 bp, respectively. For COI, the full alignment was 602 bp, although some sequences were shorter and trimmed to 564 bp. Similarly, RS alignments were 669 bp in length, while the reduced dataset was 427 bp; *rbc*L alignments were 932 bp, with a reduced dataset of 522 bp.

Table 4. Number of DNA sequences generated for each genomic marker and locality. *Eisenia* from Desventuradas Islands, Chile, is considered as a species not a priori determined.

Species/Area	**Locality**	**Genomic compartment**				
		Mitochondrial			**Chloroplast**	
		ATP8S	**COI**	**WI**	**RS**	***rbc*L**
E. cokeri (Peru)	Lobos de Tierra I.	15	6	15	6	6
	Casma	15	5	14	5	5
	Paracas	15	5	15	5	5
	Rancherío	7	10	15	5	5
Eisenia (Desventuradas Is.)	Desventuradas Is.	14	6	14	6	6
E. gracilis (Peru)	Marcona	12	8	15	8	8
	Ilo	14	5	15	5	5
Total number of specimens		85	34	88	35	35

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The concatenated molecular dataset yielded a well-supported phylogeny under both Bayesian inference (BI) and maximum likelihood (ML) (Figure 5). The genera *Ecklonia* and *Eisenia* were recovered as strongly supported clades; within *Eisenia*, *E. arborea* was clearly segregated from the remaining taxa. The Peruvian and Chilean *Eisenia* exhibited low overall genetic diversity. Across all markers, the number of haplotypes ranged from three to five, with haplotype diversity (H) values spanning 0.629–0.732 and nucleotide diversity (π) ranging from 0.003 to 0.026 (Table 5). No haplotypes were shared among *E. cokeri* and *E. gracilis* from Peru, nor with the *Eisenia* population from the Desventuradas Islands. In contrast, *E. gracilis* exhibited no haplotype variation across the two sampled localities, regardless of the marker analyzed. A similar pattern was observed in *E. cokeri* from Peru, with a single haplotype across three localities, except for the WI marker, where all individuals from the northernmost population at Lobos de Tierra Island possessed a private haplotype distinct from that observed in the other sites. Finally, *Eisenia* from the Desventuradas Islands harbored two haplotypes for WI and ATP8S, but only a single haplotype across the remaining markers.

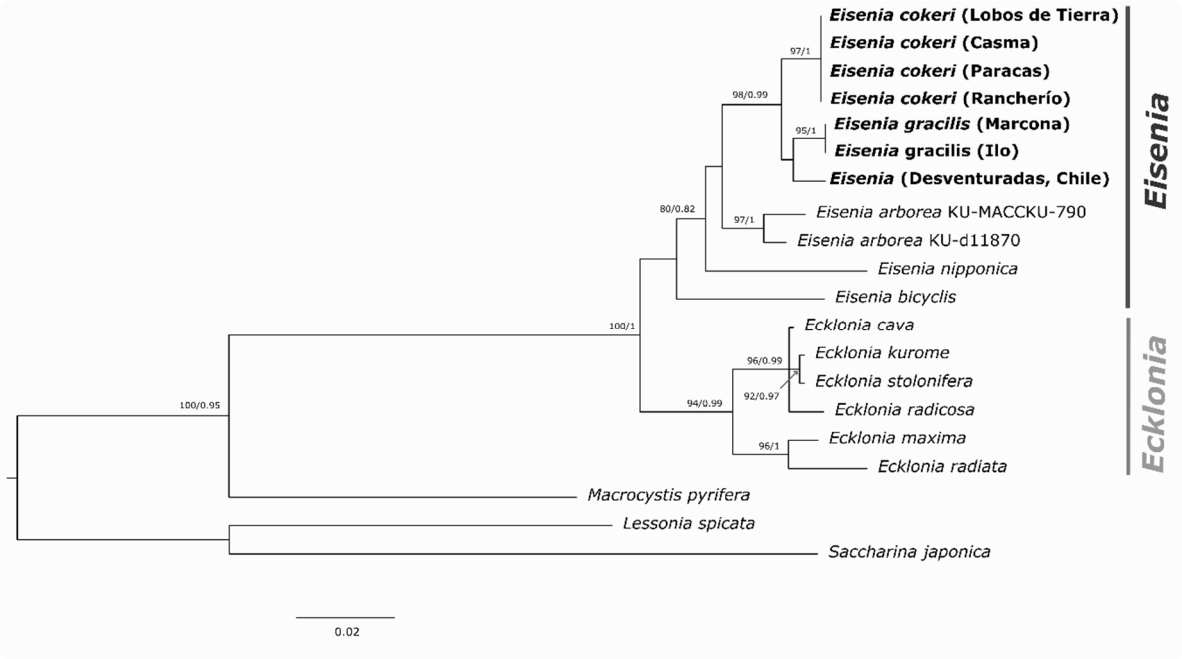

Figure 5. Phylogenetic tree of *Eisenia* DNA sequences constructed from concatenated markers. Support values were calculated using ML (bootstrap values on the left) and BI (posterior probabilities on the right) methods. Displayed are only those values exceeding the thresholds of 70% for ML and 0.80 PP.

Inferences regarding the phylogenetic relationships among *E. cokeri*, *E. gracilis*, and *Eisenia* from the Desventuradas Islands are constrained by the overall low genetic diversity. In the concatenated phylogeny (Figure 5), haplotypes of *E. gracilis* and the Desventuradas *Eisenia* formed a closer association than those of *E. cokeri*. However, analyses based on individual markers yielded incongruent topologies, complicating the interpretation of interspecific relationships (Figure S1–S5).

The reconstructed haplotype networks (Figure 6) revealed no haplotype sharing among *E. cokeri*, *E. gracilis*, and *Eisenia* from the Desventuradas Islands, supporting their genetic distinctiveness. Nevertheless, the inferred evolutionary affinities varied among markers. In the *rbc*L network, Desventuradas haplotypes were positioned equidistantly between *E. cokeri* and *E. gracilis*. In contrast, they clustered more closely with *E. gracilis* in the ATP8S, COI, and WI networks, whereas in the RS network, they were more closely associated with *E. cokeri*.

 doi:10.20944/preprints202512.0389.v1

Table 5. Genetic diversity of *Eisenia* at the species or species group level (*Eisenia* from Desventuradas Islands, Chile, is considered as a species not a priori determined), for ATP8S, COI, WI, RS and *rbc*L markers. Complete alignments are considered.

Marker	ATP8S					COI				
Group	*E. cokeri* (Peru)	*E. gracilis* (Peru)	*E. cokeri* + *E. gracilis* (Peru)	*Eisenia* (Desv.)	Total *Eisenia*	*E. cokeri* (Peru)	*E. gracilis* (Peru)	*E. cokeri* + *E. gracilis* (Peru)	*Eisenia* (Desv.)	Total *Eisenia*
bp	162	162	162	162	162	602	602	602	602	602
N	45	26	71	14	85	16	13	29	6	34
Nhap	1	1	2	2	4	1	1	2	1	3
Nhpriv	1	1	2	2	4	1	1	2	1	3
S	0	0	9	2	11	0	0	2	0	4
Ssubst	0	0	2	2	4	0	0	2	0	4
Sindels	0	0	7	0	7	0	0	0	0	0
Nindels	0	0	1	0	1	0	0	0	0	0
H	0	0	0.471	0.143	0.610	0	0	0.512	0	0.629
H (SD)	0	0	0.032	0.119	0.033	0	0	0.031	0	0.042
π	0	0	0.006	0.176	0.006	0	0	0.002	0	0.003
π (SD)	0	0	4E-04	0.001	4E-04	0	0	1E-04	0	3E-04
Π	0	0	0.942	0.286	0.981	0	0	1.025	0	1.544

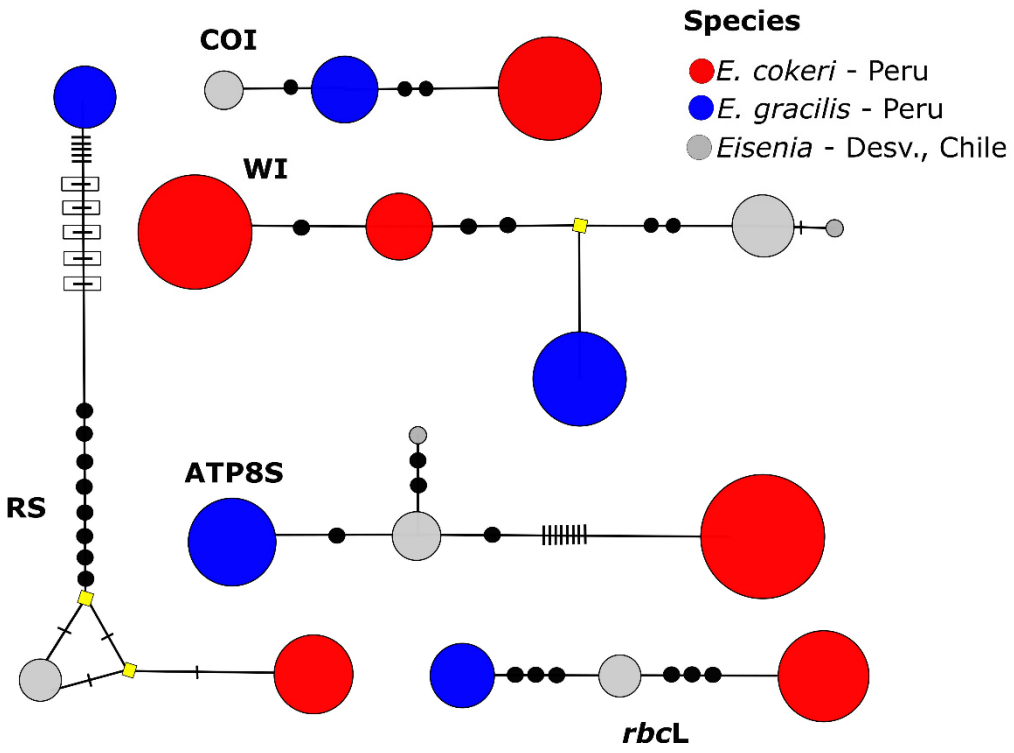

Figure 6. Haplotype networks for *Eisenia* based on DNA sequences obtained in this study for the markers ATP8S, COI, WI, *rbc*L, and RS. Circle size is proportional to the number of individuals per haplotype, and pie-chart colors indicate species assignment (red: *E. cokeri* [Peru]; blue: *E. gracilis* [Peru]; gray: *Eisenia* [Desventuradas Islands]). Line connecting haplotypes represent mutational pathways: circles along the lines denote single nucleotide substitutions, perpendicular bars indicate indels, and boxed bars correspond to blocks of 10 tandem indels. Unlabeled yellow nodes represent inferred intermediate haplotypes not detected in the sampled populations.

The interspecific divergences among our sequences of *E. cokeri*, *E. gracilis*, and *Eisenia* from the Desventuradas Islands consistently exceeded the minimum genetic distance threshold established for Laminariales (Concatenated > 0.0024; ATP8S > 0.0030; COI > 0.0036; WI > 0.0154; RS > 0.0035; *rbc*L

 doi:10.20944/preprints202512.0389.v1

> 0.0025). The highest levels of divergence were observed between *E. cokeri* and *E. gracilis* (Concatenated = 0.0260; ATP8S = 0.1148; COI = 0.0336; WI = 0.0697; RS = 0.0157; *rbc*L = 0.0012) (Table S5). In contrast, both species exhibited lower pairwise distances than *Eisenia* from the Desventuradas Islands.

4. Discussion

The integration of morphological and molecular approaches to address, for the first time, the taxonomic uncertainties of *Eisenia* species in the southeastern Pacific allowed us to confirm the distinction between two *Eisenia* species from Peru, *E. cokeri* and *E. gracilis*, described by Howe [14] and Dawson et al. [16], respectively. Furthermore, our molecular findings resolved the ambiguity between *E. cokeri* and the Northeast Pacific species *E. arborea* and suggest that *Eisenia* from the Desventuradas Islands, Chile, represents a new species. This study establishes three distinct endemic species in the region: two in Peruvian mainland waters and one in oceanic Chilean islands. These results lay the groundwork for the management and conservation of these foundational species.

4.1. Morphological Phylogenetic Position of Eisenia within Laminariales

Previous molecular studies consistently reported a close phylogenetic relationship between the laminarian genera *Eisenia* and *Ecklonia* [4,9–11] (Saunders & Druehl 1993, Yoon & Boo 1999; Yoon et al. 2001, Lane et al. 2006), to the point of suggesting that both might constitute a single genus. This hypothesis was reinforced by the limited morphological differentiation between them, with the main diagnostic trait being the pattern of meristem division at the distal end of the stipe [12]. More recently, Kawai et al. [13] rejected the proposed synonymy and reinstated *Eisenia* as a genus distinct from *Ecklonia*, although they did not include representatives from the southeastern Pacific.

Our concatenated phylogeny resolves two strongly supported clades corresponding to *Ecklonia* and *Eisenia*, confirming the placement of southeastern Pacific taxa within *Eisenia* and aligning with previous molecular studies (e.g., Kawai et al. [13], Buglass et al. [19]). Nevertheless, further sequencing efforts, especially phylogenomic datasets and the inclusion of additional Pacific species such as *E. galapagensis*, *E. desmarestioides*, and *Eisenia* sp., would refine our understanding of intergeneric boundaries and clarify species-level relationships within *Eisenia*.

4.2. Resolving the E. cokeri–E. arborea Ambiguity

Our results also clarify the long-standing taxonomic uncertainty between *E. cokeri* from Peru and *E. arborea* from North America. Historically, their high morphological similarity led several authors to question whether they represented distinct species. Howe [14], in the original description of *E. cokeri*, and Dawson et al. [16] both suggested a possible conspecificity, noting that the only diagnostic difference was the presence of muciferous canals in *E. arborea*. Later, specimens from the Desventuradas Islands (Chile), initially identified as *E. cokeri*, were also hypothesized to correspond to *E. arborea* [17]. Our phylogenetic analyses clearly separate *E. arborea* from both *E. cokeri* (Peru) and the Desventuradas lineage, providing the first molecular evidence to reject their synonymy and confirming that they represent independent evolutionary entities.

4.3. How Many Species Are in the Southeast Pacific? Evidence from Molecular Delimitation

Eisenia gracilis and *E. cokeri* were traditionally differentiated solely through morphology [16,17]. However, delimiting species based on genetic data can be challenging, particularly in recently diverged lineages or in cases involving incomplete lineage sorting, hybridization, or introgression [44]. In such situations, congruence among multiple lines of evidence—monophyly in phylogenetic reconstructions, species delimitation models (e.g., GMYC), and multilocus datasets—is generally required to support species boundaries [45,46]. Despite its usefulness, GMYC may be sensitive to incomplete sampling, variation in effective population size, or the presence of rare taxa [47].

 doi:10.20944/preprints202512.0389.v1

In our analysis, three independent genetic entities (*E. cokeri*, *E. gracilis*, and *Eisenia* from the Desventuradas Islands) were consistently recovered across molecular delimitation methods, with two of them (*E. cokeri* and *E. gracilis*) also showing distinct morphological traits. Although the genetic divergence between *E. cokeri* and *E. gracilis* is relatively low for COI (0.36%) and *rbc*L (0.73%), these values still exceed the intraspecific divergence observed within each species and fall within thresholds reported for species-level differentiation in other kelps. For comparison, COI divergence between *Eisenia* species ranges from 2.37 to 3.12% [48], and *rbc*L divergence between *Ecklonia radiata* and *Ecklonia maxima* ranges from 3 to 7% [15].

The resolving power of molecular markers varies among studies and low divergence may obscure species boundaries. Rothman et al. found that *E. bicyclis* and *E. arborea* did not form monophyletic clades when *rbc*L or ITS was used, likely because these markers are not highly informative phylogenetically [12]. Conversely, more informative loci (e.g., atp8–trnS and WI) resolve *Eisenia* relationships more clearly in concatenated datasets. Likewise, Kawai et al. demonstrated that single-marker analyses could not distinguish *E. nipponica*, whereas multilocus datasets strongly supported its species-level status [13]. Therefore, additional markers with higher polymorphisms, such as SNPs or microsatellites, would further increase resolution and robustness in delimiting *Eisenia* species [49–51]. Across phylogenetic trees, haplotype networks, and pairwise genetic distances, *E. cokeri*, *E. gracilis*, and *Eisenia* from Desventuradas consistently formed separate and well-supported evolutionary lineages, with no haplotype sharing across markers. Pairwise divergences exceeded the minimum threshold for interspecific differentiation in Laminariales, and lineage-specific mutations (e.g., indels) indicate restricted or absent gene flow, particularly in the Desventuradas lineage. Although this lineage clearly represents an independent evolutionary unit, confirming its species status will require additional data—ideally, genome-wide SNPs—to test this delimitation under an integrative framework.

The Desventuradas Islands are located ~1300 km from the southernmost Peruvian population and are separated from continental Chile by an oceanographic barrier driven by contrasting temperature and salinity gradients [52]. Their biota are known for high endemism and Indo-Pacific affinities [43,53]. Taken together, the molecular, morphological, and biogeographical evidence indicates the presence of a previously unrecognized species of *Eisenia* endemic to the Desventuradas Islands and supports restricting *E. cokeri* as an endemic species of Peruvian coastal waters.

4.4. Morphological and Morphometric Distinctiveness

The morphological characterization of *E. cokeri* and *E. gracilis* presented here refines and expands upon the original taxonomic descriptions [14,16] by providing quantitative measurements of external structures, including total thallus length, holdfast dimensions, and blade size. Adult sporophytes of the two species exhibit marked differences in overall morphology (Figures 2 and 3). *E. cokeri* develops a robust thallus with a well-formed holdfast, stipe, and blades, whereas *E. gracilis* forms smaller sporophytes characterized by a rhizomatous holdfast, cord-like stipe, and narrow, single-lamina blades. Additional diagnostic traits—such as stipe differentiation (longitudinal divisions, pseudostipe formation), degree of frond branching, margin serration, and surface roughness—further differentiate the two species.

Although seaweed systematics increasingly relies on molecular phylogenetics, the quantitative assessment of morphological traits remains a critical component for supporting phylogenetic hypotheses and enabling reliable field identification [54,55]. Our morphometric comparison strongly supports the distinction between the two Peruvian species: *E. cokeri* is significantly larger and exhibits a more complex architecture, while *E. gracilis* is consistently smaller, slender, and lightweight. The PCA revealed distinct morphometric clusters for each species and highlighted greater intraspecific variation in *E. cokeri*. Individuals from Lobos de Tierra Island were generally smaller—with shorter thalli, shorter stipes, and smaller conical holdfasts—than those from Casma and Paracas, yet still exhibited higher biomass than the Paracas plants. Conversely, individuals from Casma displayed the largest measurements across all traits. This morphological variability within *E. cokeri* is consistent

 doi:10.20944/preprints202512.0389.v1

with its occurrence in shallow, highly dynamic environments, where temperature fluctuations, wave exposure, and substrate instability can promote phenotypic plasticity. In contrast, the more uniform morphology of *E. gracilis* likely reflects the comparatively stable conditions of deeper subtidal habitats.

4.5. Spatial and Ecological Segregation

Our sampling of *E. cokeri* covered nearly its entire known latitudinal range (6°–26° S), including northern and central Peru (Lobos de Tierra, Casma, Paracas) and the Desventuradas Islands (Chile). Its distribution along the Peruvian coast appears stable since its original description [14], although it is no longer present in historical locations such as Callao and Pucusana (12° S). For *E. gracilis*, we confirm its presence in Marcona—the type locality and the only site previously reported [16]—and extend its distribution southward to Ilo (17° S). Together, these results reveal a disjunct latitudinal pattern: *E. cokeri* predominates in northern and central Peru (up to ~14° S), whereas *E. gracilis* inhabits central and southern Peru, with its northern limit around 15° S.

Bathymetric distribution further distinguishes the species. *Eisenia cokeri* occurs in the lower intertidal and shallow subtidal (0–10 m), while *E. gracilis* occupies deeper subtidal habitats (10–30 m). *Eisenia* from the Desventuradas Islands forms beds from 0 to 34 m [17]. These depth patterns align with the ecological breadth reported for the genus, which includes species restricted to shallow waters (<12 m) and others extending to mesophotic depths (up to 60 m). Depth partitioning may reflect the interplay of light availability and interspecific competition [56,57], with deeper habitats (>10 m) likely reducing substrate competition with kelps such as *Macrocystis pyrifera* and *Lessonia trabeculata*. Given the scarcity of depth records for *E. gracilis*, its occurrence below 30 m suggests the potential presence of deep-water kelp forests, a habitat type proposed for this region by Graham et al. [23].

Eisenia from the Desventuradas Islands, previously misidentified as *E. cokeri*, shows consistent morphological differences—including holdfast shape and a wider, compressed stipe—and narrower, lanceolate blades [27]. Its occurrence in highly exposed, high-energy environments suggests that stipe thickening may be an adaptation to hydrodynamic stress, as reported for other kelps.

5. Conclusion

This study resolves long-standing taxonomic uncertainties within *Eisenia* in the southeastern Pacific. By integrating multilocus phylogenetic analyses, molecular species delimitation, detailed morphology, and ecological (spatial and depth) segregation, we demonstrate that *E. cokeri* and *E. gracilis* represent two distinct species in Peruvian coastal waters. At the same time, the lineage from the Desventuradas Islands in Chile constitutes a third, previously unrecognized evolutionary unit. Phylogenetic analyses separate this oceanic lineage and *E. cokeri* from the North American species *E. arborea*, rejecting historical assumptions of conspecificity. Morphological and morphometric analyses corroborate these findings by revealing clear diagnostic differences between species, and the contrasting depth and latitudinal distributions reflect ecological partitioning across environments, from shallow, hydrodynamically variable habitats (*E. cokeri*) to deeper mesophotic zones (*E. gracilis*), with the Desventuradas lineage adapted to exposed oceanic conditions.

Together, genetic, morphological, and ecological evidence converge to consistently support the existence of three independent species of *Eisenia* in the southeastern Pacific—two endemic to Peru and one endemic to the Desventuradas Islands—highlighting previously unrecognized regional biodiversity and providing a foundation for future conservation efforts.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Phylogenetic tree constructed from RS marker. Support values were calculated using ML (top) and BI (bottom) methods. Displayed are only those values exceeding the thresholds of 70 for ML and 0.80 BI, and the asterisk (*) denotes values falling below these specified minimum thresholds; Figure S2: Phylogenetic tree constructed from WI marker. Support values were calculated using ML (bootstrap

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values on the left) and BI (posterior probabilities on the right) methods. Displayed are only those values exceeding the thresholds of 70 for ML and 0.80 BI and the asterisk (*) denotes values falling below these specified minimum threshold; Figure S3: Phylogenetic tree constructed from COI marker. Support values were calculated using ML (bootstrap values on the left) and BI (posterior probabilities on the right) methods. Displayed are only those values exceeding the thresholds of 70% for ML and 0.80 PP and the asterisk (*) denotes values falling below these specified minimum thresholds; Figure S4: Phylogenetic tree constructed from *rbc*L marker. Support values were calculated using ML (bootstrap values on the left) and BI (posterior probabilities on the right) methods. Displayed are only those values exceeding the thresholds of 70% for ML and 0.80 PP for BI; Figure S5: Phylogenetic tree constructed from ATP8S marker. Support values were calculated using ML (bootstrap values on the left) and BI (posterior probabilities on the right) methods. Displayed are only those values exceeding the thresholds of 70 for ML and 0.80 BI and the asterisk (*) denotes values falling below these specified minimum thresholds; Table: S1. Morphometric data for *Eisenia cokeri* and *Eisenia gracilis* obtained from previous research conducted in 2009 and 2015 (P. Carbajal, unpublished data); Table S2: Molecular markers, primers, and PCR protocols used for PCR reactions and DNA sequencing. The genomic compartment of origin, the abbreviations used in this study, and the full name of each marker are provided, along with the name, sequence, and bibliographic reference of each partition. In addition, references to the protocols specifying reagent concentrations and PCR cycling conditions are included; Table S3. Specimens of GenBank included in the molecular analysis with their accession numbers. '*' indicates the sequences that were used for the concatenated tree, '-' indicates sequence not available in GenBank. Each species of *Ecklonia* and *Eisenia* was used for GMYC analyses; Table S4. Mann–Whitney tests of morphometric variables of *Eisenia cokeri* and *E. gracilis* measured in five locations along the Peruvian coast; Table S5. Genetic distances for species of *Eisenia*, *Ecklonia*, and the *Macrocystis pyrifera*. The average number of nucleotide base differences per site between species sequences is shown. 'n/c' means not calculated.

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