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Unraveling Plecoptera Diversity in Two Protected Areas of Argentine Patagonia

TÁCIO DUARTE, GABRIEL M. MARTIN, DANIELLE ANJOS-SANTOS & PABLO PESSACQ

Abstract: The Plecoptera taxonomy in Patagonia is well-documented, yet their distribution remains poorly understood, hindering comprehensive ecological and biogeographical studies. This study enhances knowledge of stonefly distribution in two Patagonian national parks: Nahuel Huapi and Los Alerces. Extensive fieldwork, georeferenced species records, and geographic information system data integration were conducted. Species richness was calculated using polygons (0.1° x 0.1° pixels) across ecoregions, with species indexed from rare to ubiquitous. Cluster analyses revealed faunal affinities across ecosystem complexes, and richness estimators (Jack1, Jack2, and Chao2) highlighted knowledge gaps. Results showed uneven species distribution, with the highest richness polygon (n = 19) in Los Alerces. The Northern Moist Forests hosted the most species, followed by the Transitional Cypress-Beech Forests. The rarest species were also found in these two complexes, as well as the Ecotone Steppe-Forest. Cluster analysis revealed strong affinities between the Northern Moist Forests of Nahuel Huapi and Ecotone Steppe-Forest. Richness estimators suggested up to 23 undocumented species. Though much remains to be learned about Plecoptera distribution in Patagonia, this study emphasizes the critical role of national parks in conserving biodiversity and provides a foundation for future conservation strategies, identifying new taxa records, including southernmost distributions.

Key words: distribution, geographic coordinates, Argentine National Parks, stoneflies, taxonomy.

INTRODUCTION

Effective conservation measures often face a major challenge known as the Wallacean shortfall, referring to the lack of comprehensive knowledge about species distributions (Lomolino 2004). Overcoming this hurdle requires the acquisition of accurate and precise distribution records, which also play a crucial role in correlating environmental predictors such as temperature, latitude, altitude, precipitation, etc., with taxa distribution (Diele-Viegas 2021). This essential information enables the assessment of changes in species distribution, encompassing range disappearances, expansions, or declines, which are in response to thermal impacts resulting from climate change and other influential factors.

Particularly, the warming of continental aquatic habitats can pose significant challenges for numerous aquatic insect species, in particular the stoneflies (Order Plecoptera) (Frakes et al. 2021), a group known for their specific temperature and oxygen requirements (Stark et al. 2009). These insects play a crucial role in freshwater ecosystems, as they act as important links in trophic transfers and contribute to vital ecosystem services such as organic matter processing and nutrient cycling (Stewart &

Stark 2002). The sensitivity of stonefly larvae makes them integral to aquatic biomonitoring systems (Cheney et al. 2019). The presence and abundance of adult stoneflies, typically found in riparian vegetation or on rocks near streams, are greatly influenced by habitat degradation and loss of aquatic environments (Hynes 1976).

Aquatic insects from Argentine Patagonia are relatively well known, as evidenced by recent revisions and taxonomic updates including them (e.g. Archangelsky et al. 2023, Lozano et al. 2023, Michat et al. 2023, Pessacq & Duarte 2023, Sganga et al. 2023). The taxonomy of Plecoptera is well known, with six families, 38 genera, and 82 species found in Argentina. From these species, 46 in 33 genera have been recorded in Argentine Patagonia (Pessacq & Duarte 2023). Our understanding of Patagonian stoneflies is mainly due to the extensive studies of J. Illies, conducted during the mid-20th century (e.g. Illies 1958, 1960, 1963, 1965, 1969) and the recent works of several authors, who described many novelties and conducted phylogenetic analyses (e.g. Vera 2006a, b, 2007, 2008, 2009, 2016, 2019, McLellan & Zwick 2007, Pessacq 2008, 2009, Pessacq & Omad 2012, Pessacq & Rivera-Pomar 2019, Pessacq et al. 2020). Although these studies played an important role in starting the compilation of species basic data, information on the group's distribution is still in its early stages. In most cases, information on the species' chorology is limited to the original description and isolated citations, creating a significant barrier to the group's distributional, ecological, and biogeographical research (Pessacq & Duarte 2023). Furthermore, some of its taxa have a significant lack of information on immature stages, and new species are expected to be described (Pessacq & Duarte 2023).

Plecoptera, unlike other insects, is considered to be more diverse at higher latitudes (DeWalt & Ower 2019). Although this is true at the family and generic levels, the Neotropical region is likely home to the greatest number of species worldwide (Pessacq & Duarte 2023).

Palma & Figueroa (2008), based on literature, investigated the distribution of stonefly diversity across Chilean Patagonia and discovered that the order's diversity is highest in the Valdivia region (between -39° and -40°) and dramatically decreases at -42° to -43°, with a second peak between -50° and -54°, showing a bimodal pattern. Muzón (1997) found a similar result for Odonata, with a higher number of species at -40° and a sudden decrease at -42° to -43°. Valdovinos et al. (2010), on a study based on original fieldwork data and focused between -42.83° and -54.72°, found a latitudinal change in the stonefly diversity, with a gradient of decreasing species richness towards the south, probably due to the input of solar energy and its effect on species diversity. It should be noted that Patagonian dragonflies have a stronger influence from the Neotropical region fauna (Muzón et al. 2014), while stoneflies have a stronger relation with fauna from southern regions (i.e. Australia, New Zealand) (Pessacq & Duarte 2023).

The study of Plecoptera latitudinal distribution in Patagonia is still in its early stages, and research on their distribution at the ecoregion level remains nonexistent. The general objective of this work is to deepen our understanding of the geographical distribution of stonefly fauna in Argentine Patagonia, focusing on two national parks (Nahuel Huapi and Los Alerces) that differ in latitude, size, and presence of ecoregions and ecosystem complexes. We aim to estimate and compare the stonefly species richness for the region and analyze the relationship between different ecosystem complexes and the stonefly species recorded in these areas. Given the differences between both national parks, we hypothesize that stonefly

richness will be lower in Los Alerces National Park compared to Nahuel Huapi National Park. Moreover, we expect to find differences in stonefly fauna across the different ecosystem complexes and between national parks.

Given the complete lack of information regarding the association of stoneflies with different geographic and climatic units (ecoregions and ecosystem complexes) in Patagonia, we aim to obtain the first data that will allow us to understand the existence of these relationships.

We include precise species identification and accurate geolocation based on research projects conducted between 2007 and 2022, as well as a comparison of the two selected national parks. This first step allows the analysis of richness distribution in the selected areas, the identification of the ecoregions and ecosystem complexes where the different species occur, and the classification of which species can be considered as rare or ubiquitous. So far, we do not know which aquatic insect species are rare or found in high-risk environments. Whenever possible, potential impacts associated with the presence of the different stonefly species will be identified.

Finally, we provide a comprehensive species list, new records, and updated taxonomic information at the generic and familiar levels from both national parks. The species list will be valuable for future monitoring of changes in the Plecoptera fauna, which may result from anthropogenic or natural factors, a task currently challenging due to the limited knowledge of their distribution.

MATERIALS AND METHODS

Study area and climatic conditions

The study area comprises two national parks of northwestern Argentine Patagonia: Nahuel Huapi (NHNP, Fig. 1) and Los Alerces (LANP, Fig. 2). The first one is the largest one and the first national park of Argentina, with a protected area of 717,261 ha, located between coordinates South -41° and West -71.5° in Neuquén and Río Negro Provinces. Los Alerces National Park covers 259,822 ha in Chubut Province, located at South -42.8° and West -71.8°. Aside from size differences (NHNP is 2.8 times larger than LANP), NHNP is located between latitudes -40.14° and -41.60°, near the latitude (between -39° and -40°) with the highest species richness of Plecoptera observed by Palma & Figueroa (2008) in Chile, while LANP is located between latitudes -42.55° and -43.18°, where the species richness curve drops significantly, reaching one of its lowest values at -43° (Palma & Figueroa 2008). The aforementioned is coupled with an expected reduction in species richness along the N-S gradient (Valdovinos et al. 2010). Furthermore, both national parks are composed by distinct ecosystem complexes; NHNP has two ecoregions and four ecosystem complexes, whereas LANP has one ecoregion and three ecosystem complexes (see Patagonian regionalization and distribution analysis, as well as Figs. 1 and 2).

Northwestern Patagonia has a temperate-cold climate (Paruelo et al. 1999), characterized by a steep precipitation gradient from west to east and a moderate temperature gradient. The Andes mountains on the western side of South America disrupt the movement of humid air masses from the Pacific Ocean, resulting in most precipitation occurring on the west side of the mountains, characterized by forests and moorlands. In contrast, the eastern side of the Andes in Patagonia experiences drier and warmer conditions (Labraga & Villalba 2009), and moving further to the east, the vegetation changes to steppes, semi-deserts, and shrublands (Villagrán & Armesto 2005). Annual precipitation ranges from ~4,000 mm in the Valdivian Rainforest to

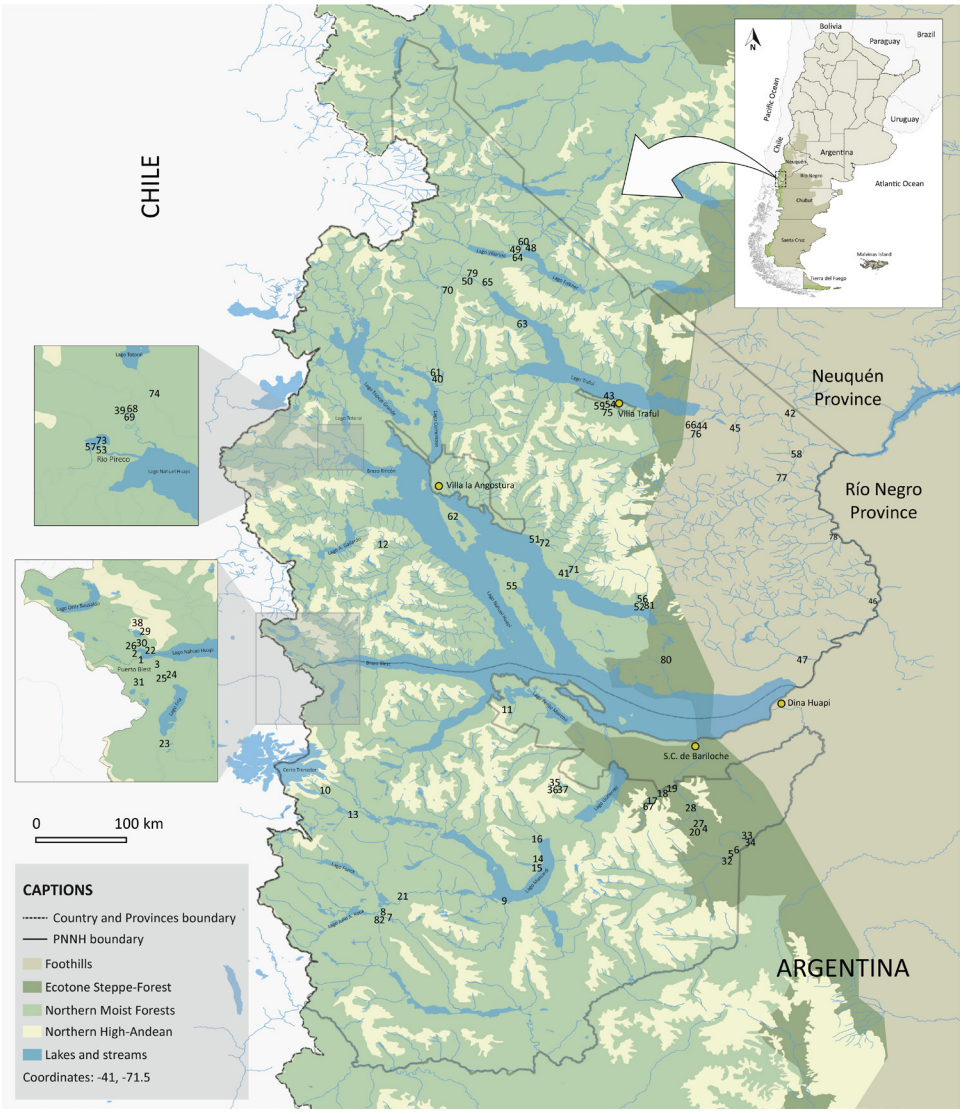

Figure 1. Map of Nahuel Huapi National Park, Argentina, indicating collection sites (numbers) and ecosystem complexes.

~230 mm in the Patagonian Steppe (Prohaska 1976), with elevations ranging from 500 to 3,000 m. These extremes occur within less than 150 km from west to east, resulting in some of the most abrupt biotic transitions known (Quintanilla 1983, Veblen & Lorenz 1988). Aside from the longitudinal gradient, there is a latitudinal gradient, with temperatures decreasing towards the south.

Specimens sampling and curation

The sampling was conducted from 2007 to 2022 in 144 sites, with 103 in NHNP and 41 in LANP. Plecoptera were found in 111 of these sites, including 82 in NHNP (Fig. 1, Table I) and 29 in LANP (Fig. 2, Table I). The list of species and sites is presented in Table II.

Fieldwork was conducted with three main grants (see acknowledgements) as well as the financial resources of the authors. Fieldwork focused on taxonomy and was conducted opportunistically and unsystematically. Consequently, the information obtained is of a presence-absence type. Due to the basic lack of distributional and taxonomic knowledge, the objective of the sampling was to cover as much

Figure 2. Map of Los Alerces National Park, Argentina, indicating collection sites (numbers) and ecosystem complexes.

surface area and as many sampling points as possible.

Adults were collected using Malaise and light traps, as well as direct capture with aerial nets. The placement of the Malaise and light traps aimed to cover areas with greater species richness or rare species, observed in preliminary fieldwork, and included remote areas with a low risk of the traps being removed or stolen. Larvae were collected with D-nets and survey nets. All specimens were fixed in 96% ethanol and are housed in the entomological collection of the Centro de Investigación Esquel de Montaña y Estepa Patagónica (CIEMEP-CONICET), Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), in Esquel, Chubut.

Species identification and rarity

The specimens studied were examined using a Leica S9E stereomicroscope. Species were identified using generic keys (Pessacq & Duarte 2023), original descriptions (e.g., Illies 1958, 1960, 1963, 1964, 1969, Vera 2008, 2012), and comparisons with species in the CIEMEP collection.

Despite recent advances in the knowledge of Patagonian stoneflies, the immature

Table I. Plecoptera collection sites and their respective decimal degrees in Nahuel Huapi (82 sites) and Los Alerces (29 sites) National Parks, Argentina.

Sites in Nahuel Huapi National Park					
(Site number) Site name	**Latitude**	**Longitude**	**(Site number) Site name**	**Latitude**	**Longitude**
(1) Blest, temporary St.	-41,025417	-71,822611	(42) Leones St.	-40,670121	-71,156312
(2) Blest, Piedras Falls	-41,019333	-71,825750	(43) Blanco St.	-40,650833	-71,412500
(3) Blest, Pozón Alegre	-41,029222	-71,810472	(44) Minero R.	-40,691389	-71,291389
(4) Ñirihuau R.	-41,258333	-71,285000	(45) Meri St.	-40,695000	-71,232500
(5) Ñirihuau R.	-41,293222	-71,241250	(46) Corral St.	-40,935556	-71,039722
(6) Ñirihuau R.	-41,289583	-71,235694	(47) Chacabuco St.	-41,014167	-71,144444
(7) Manso medio R. 1	-41,381083	-71,731945	(48) Falkner Lk.	-40,436667	-71,528889
(8) Alerce St.	-41,381694	-71,736056	(49) Filuco St.	-40,439444	-71,536389
(9) Mascardi Lk.	-41,360972	-71,562222	(50) Pichitraful R.	-40,480000	-71,612222
(10) Blanco St.	-41,205000	-71,820556	(51) Ragintuco R.	-40,853056	-71,518056
(11) Blanco St.	-41,092861	-71,561972	(52) Pedregoso R.	-40,940833	-71,369722
(12) Manso superior R.	-40,853222	-71,736833	(53) Pireco R. 1	-40,725833	-71,800278
(13) Manso medio R. 2	-41,235583	-71,782917	(54) Blanco St.	-40,651111	-71,412222
(14) Llum St.	-41,300833	-71,510833	(55) Turbina St.	-40,940833	-71,369722
(15) Llum St.	-41,316333	-71,511056	(56) Pedregoso R.	-40,941111	-71,369722
(16) Tuqueco St.	-41,269861	-71,515278	(57) Pireco R. 2	-40,726111	-71,800833
(17) Ñireco St.	-41,217306	-71,356389	(58) Leones St.	-40,730278	-71,150833
(18) Ñireco St.	-41,204361	-71,341972	(59) Blanco St.	-40,650833	-71,412222
(19) Ñireco St.	-41,197750	-71,327917	(60) Filuco St.	-40,439722	-71,536389
(20) Challhuaco R.	-41,261639	-71,297000	(61) Neuquenco St.	-40,621667	-71,664444
(21) Manso medio R. 3	-41,354444	-71,707583	(62) Hua Huan St.	-40,817778	-71,633386
(22) La Araña St.	-41,02175	-71,818056	(63) Filuco St.	-40,540000	-71,536250
(23) Frias R.	-41,087472	-71,805528	(64) Villarino Lk.	-40,445972	-71,545444
(24) Las Turberas St.	-41,040000	-71,804028	(65) St. nº1 in Villarino	-40,482722	-71,584694
(25) Grande St.	-41,039333	-71,807500	(66) Minero R.	-40,689722	-71,292778
(26) La Heladera “mallin”	-41,015556	-71,829167	(67) Ñireco R.	-41,217583	-71,356389
(27) Los Patos “mallin”	-41,263333	-71,297222	(68) Totoral St.	-40,712861	-71,789917
(28) Challhuaco R.	-41,225806	-71,297167	(69) Pichitraful R.	-40,712806	-71,789944

Table I. Continuation.

(29) Cántaros Lk.	-41,006389	-71,821944	(70) Falls in Pichitraful R.	-40,497278	-71,645556
(30) Cántaros Lk.	-41,018889	-71,824167	(71) Huemul St.	-40,896167	-71,472778
(31) Los Clavos St.	-41,040278	-71,824444	(72) Ragintuco R.	-40,853222	-71,518056
(32) Ñirihuau R.	-41,298611	-71,253056	(73) Pireco R. 3	-40,726222	-71,800833
(33) Botella St.	-41,271944	-71,221111	(74) St. nº 2 in Villarino	-40,707278	-71,780556
(34) Botella St.	-41,271389	-71,215833	(75) Blanco St.	-40,651138	-71,412444
(35) Schmoll Lagoon	-41,193333	-71,497500	(76) Minero R.	-40,691139	-71,291583
(36) Vantitter St.	-41,198056	-71,496389	(77) Cuyin Manzano R.	-40,763333	-71,170833
(37) Tonke Lagoon	-41,198333	-71,486667	(78) Carbón St.	-40,843583	-71,096667
(38) La Heladera St.	-41,000833	-71,824167	(79) Pichitraful R.	-40,480139	-71,612250
(39) Totoral St.	-40,712583	-71,790556	(80) Castilla St.	-41,019028	-71,339389
(40) Neuquenco St.	-40,621667	-71,664167	(81) Pedregoso St.	-40,941222	-71,369917
(41) Huemul St.	-40,896389	-71,472778	(82) Alerces waterfall st.	-41,381639	-71,735889
Sites in Los Alerces National Park					
(Site number) Site name	**Latitude**	**Longitude**	**(Site number) Site name**	**Latitude**	**Longitude**
(83) St. in Verde Lk.	-42,717500	-71,727778	(98) Irigoyen St.	-42,859791	-71,602613
(84) St. in Los Maitenes Camping	-42,891944	-71,612500	(99) Krügger Lk.	-42,884289	-71,729472
(85) St. 3 south to Los Pumas	-42,877333	-71,623222	(100) Pucon Pai St.	-42,825972	-71,61225
(86) Trib. st. Arrayanes	-42,739444	-71,741944	(101) Rivadavia, Lk.	-42,825736	-71,611772
(87) Trib. st. Frey 1	-42,902778	-71,718611	(102) Verde Lk.	-42,666389	-71,681111
(88) Trib. st. Frey 2	-42,916111	-71,715833	(103) Desaguadero R.	-42,730556	-71,743889
(89) Trib. st. Frey 5	-42,927567	-71,712753	(104) Colegual St. Bridget	-42,677222	-71,686111
(90) St. 1 in Krügger-Futalaufquen	-42,879722	-71,729167	(105) Rivadavia R.	-42,673611	-71,698889
(91) Canales Doña Rosa	-42,690000	-71,703611	(106) Nalcadero St.	-42,808389	-71,648778
(92) Cisne R.	-42,609167	-71,888889	(107) Rañinto St.	-42,954167	-71,591944
(93) Colegual St.	-42,673611	-71,69500	(108) Canelo bis St.	-42,915889	-72,070639
(94) Coronado St.	-42,633056	-71,698611	(109) Negro St.	-42,702639	-71,704694
(95) Frey R. “boca”	-42,891944	-71,730556	(110) Los Pumas St.	-42,861667	-71,624167
(96) Frey R. “Palanganas”	-42,916389	-71,717500	(111) St. 1 South to Los Pumas	-42,875861	-71,624583
(97) Frey R. footway	-42,900556	-71,725556			

stages of several species and genera remain unknown or indistinguishable, making species-level differentiation impossible (Vera 2012, Pessacq & Duarte 2023). These taxa include species from the genera *Diamphipnoa*, *Diamphipnopsis*, *Austronemoura*, *Neofulla*, and *Udamocercia*, most of whose larval stages are still undescribed. Additionally, the larval stages of *Chilenoperla* species + *Pelurgoperla personata* and *Notoperla magnaspina* + *N. archiplatae* are indistinguishable (Vera 2008, 2012). Consequently, these taxa were included in the analyses only when identified in their adult stages, where specific identification is possible. Furthermore, some specimens were collected as early immature instars and cannot be identified at the species level; this is particularly evident for the few *Pictetoperla* specimens studied.

For each species, we included the record status (i.e., new provincial or national record) and, if applicable, the potential risks associated with the site where it was found. The rarity of a species or genus (Figs. 5a and 5b, as well as species lists), was determined as follows: a very rare species is recorded in 1-2 sites, a rare species in 6-12 sites, a common species in 13-20 sites, and a ubiquitous species in more than 20 sites.

Patagonian regionalization and distribution analysis

For regionalization of the studied area, we follow Morello et al. (2018), where the ecoregions are defined as geographical areas within the country characterized by distinct regional climates. These climates are determined by factors such as average annual rainfall, the presence or absence of a dry season, and the occurrence of cool or cold seasons. These ecoregions also share a common biogeographic history. Additionally, ecosystem complexes are defined as a grouping of ecological systems that tend to occur repeatedly in relation to geomorphological and edaphic units or landscapes, share climate, land use, and ecological flows, and are identified by their location within the regional relief and the phenology of the dominant formation. The national parks analyzed contain two ecoregions (Patagonian Steppe and Patagonian Forest) and five ecosystem complexes (Foothills, Northern High-Andean, Ecotone Steppe-Forest, Northern Moist Forests, and Transitional Cypress-Beech Forests). NHNP includes both ecoregions and four ecosystem complexes (i.e., Foothills, Northern High-Andean, Ecotone Steppe-Forest, and Northern Moist Forests). LANP, in turn, exclusively represents the Patagonian Forest ecoregion, along with three ecosystem complexes (i.e., Northern High-Andean, Northern Moist Forests, and Transitional Cypress-Beech Forests).

Species records were georeferenced and integrated into a geographic information system. Richness was calculated using the point-to-polygon tool in DIVA-GIS v.7.5 (Hijmans et al. 2001), with a pixel size of 0.1° x 0.1° km (equivalent to an area ranging from 93.3 km2 to 90 km2). Information on ecoregion and ecosystem complex (sensu Morello et al. 2018) was retrieved for each species' locality using the point sampling tool in QGIS 3.22.4-Białowieża (QGIS Development Team 2022).

Species richness estimates and cluster analysis

To estimate species richness, we used data on Plecoptera species from the five ecosystem complexes present in the two national parks analyzed. Nonparametric methods were used to analyze presence-absence data, with ecosystem complexes serving as sampling units. We conducted our analyses using the R environment (R Core Team 2023). These calculations were carried out with the 'specpool'

Table II. List of Plecoptera species and their respective collection sites in Nahuel Huapi (NHNP) and Los Alerces National Parks (LANP), Argentina. * Indicates identification based on larval stage.

Nr.	Species	NHNP sites	Total sites in NH	LANP sites	Total sites in LA	Total sites
AUSTROPERLIDAE						
1	*Klapopteryx kuscheli*	8, 9, 11, 15, 16, 18, 19, 20, 21, 23, 27, 28, 31, 32, 35, 36, 37, 39, 43, 44, 50, 51, 52, 53, 56, 57, 61, 63, 65, 67, 69, 71, 72, 73, 76, 77, 79, 80, 81, 82	40	87, 88, 92, 93, 98, 100, 104, 105, 106, 107, 108	11	51
2	*Penturoperla barbata*	25, 29, 31	3	100, 106	2	5
DIAMPHIPNOIDAE						
3	*Diamphipnopsis virescentipennis*	26, 29, 38, 82	4	88, 89	2	6
4	*Diamphipnoa helgae*	-	-	85	1	1
*	*Diamphipnoa* sp.	21, 31, 61, 65, 70, 82	6	-	-	6
EUSTHENIIDAE						
5	*Neuroperla schedingi*	25, 28, 38, 53, 82	5	87, 88, 89, 92, 96	5	10
GRIPOPTERYGIDAE						
6	*Alfonsoperla flinti*	-	-	100, 111	2	2
7	*Antarctoperla michaelseni*	2, 12, 19, 34, 39, 42, 44, 47, 61, 63, 67, 72, 77, 78, 79, 81	16	85, 86, 88, 91, 97, 98, 99, 100, 101, 103, 105, 109, 111	13	29
8	*Aubertoperla illiesi*	2, 7, 8, 10, 14, 15, 16, 18, 20, 21, 22, 24, 28, 29, 30, 38, 39, 41, 44, 45, 46, 49, 50, 57, 70	25	83, 85, 87, 91, 98, 100, 103, 104, 105, 109	10	35
9	*Ceratoperla fazi*	28, 33, 40, 74, 79	5	-	-	5
*	*Chilenoperla* sp. / *Pelurgoperla personata*	5, 12, 32, 33, 38, 50, 55, 58, 60, 62, 68, 74, 80, 82	14	90, 106, 109	3	17
10	*Chilenoperla elongata*	27	1	-	-	1
11	*Chilenoperla puelche*	28	1	-	-	1
12	*Ericiataperla puerilis*	-	-	98	1	1
13	*Limnoperla jaffueli*	1, 2, 4, 19, 31, 34, 45, 46, 47, 48, 58, 70, 80	13	85, 86, 91, 95, 98, 100, 101, 102	8	21
14	*Neopentura semifusca*	40, 69, 74, 79, 82	5	-	-	5
*	*Notoperla* sp.	40, 69, 74, 79	4	87, 107, 108, 110	4	8
15	*Notoperla archiplatae*	11	1	-	-	1
*	*Notoperla archiplatae* / *N. magnaspina*	19, 28, 32, 52, 54, 56, 59, 63, 72, 75, 81	11	98, 108	2	13
16	*Notoperla fasciata*	10, 11, 20, 23	4	100	1	5
17	*Notoperlopsis femina*	17	1	-	-	1

Table II. Continuation.

18	*Rhithroperla rossi*	39, 40, 50, 57, 60, 63, 64, 66, 67, 68, 71, 72, 73, 76, 81	15	94	1	16
19	*Senzilloides panguipullii*	4, 12, 15, 20, 28, 30, 54, 55, 65, 81	10	98	1	11
20	*Teutoperla brundini*	25	1	85, 87, 88, 111	4	5
21	*Uncicauda testacea*	-	-	98, 100	2	2
NOTONEMOURIDAE						
*	*Austronemoura* sp.	24, 33, 54, 57, 69, 70, 72, 74, 81	9	87, 106, 110	3	12
22	*Austronemoura chilena*	-	-	96, 98, 100	3	3
23	*Austronemoura encoensis*	25	1	-	-	1
24	*Neonemura barrosi*	13, 19, 24, 25	4	84, 85, 97, 111	4	8
*	*Neofulla* sp.	25	1	88	1	2
25	*Neofulla biloba*	-	-	98	1	1
*	*Udamocercia* sp.	2, 3, 4, 6, 19, 28, 32, 33, 50, 60, 63, 68, 69, 70, 73, 74, 79, 80	18	92, 109	2	20
26	*Udamocercia frantzi*	-	-	95, 96	2	2
PERLIDAE						
*	*Pictetoperla* sp.	-	-	92, 96, 105	3	3
27	*Pictetoperla gayi*	15, 31, 39, 61, 72	5	-	-	5

function from the R package vegan (Oksanen et al. 2022). The following richness estimators were used: 1) Chao2; 2) first-order jackknife (Jack1); and 3) second-order jackknife (Jack2) (Magurran 2004). These methods consider the number of rare species, which are defined as the number of species that occur in just one sampling unit and the number of species that occur in two sampling units (see Magurran 2004).

We used cluster analysis to determine the affinity of Plecoptera fauna across ecosystem complexes (Legendre & Legendre 2012). Cluster analysis was performed using the unweighted pair-group method with arithmetic averages (UPGMA) through the 'hclust' function from the R package vegan (Oksanen et al. 2022). In this analysis, we employed the Jaccard dissimilarity index to measure the dissimilarity between ecosystem complexes (Legendre & Legendre 2012). The Northern Moist Forests ecosystem complex was the only one common to both parks to have species recorded. Therefore, in our analysis, we separated this ecosystem complex to compare them in terms of dissimilarity. To avoid confusion, it is important to note that faunal similarity between ecosystem complexes is inversely proportional to dissimilarity.

We used the Cophenetic Correlation Coefficient (CCC) to determine whether the dendrogram was consistent with the original dissimilarity index. A CCC greater than 0.8 indicates a good fit between the dendrogram and the original dissimilarity index (Legendre & Legendre 2012). We also used the Mantel test to determine whether the dissimilarity between ecosystem complexes is related to geographic distance. This analysis utilized the 'mantel.rtest'

function from the R package vegan ADE 4 (Dray & Dufour 2007).

Challenges posed by immature specimens

It is important to note that seven taxa (*Austronemoura* sp., *Chilenoperla*/*Pelurgoperla personata*, *Diamphipnoa* sp., *Neofulla* sp., *Notoperla* sp., *Pictetoperla* sp., and *Udamocercia* sp.) could not be identified at the species level due to incomplete information on immature stages or because immatures of some species are so far indistinguishable (see Material and Methods section for details). Figures 4b and 5b do not include these unidentified taxa. As a result, some species represented only by immature specimens in our sampling that belong to widespread genera and are likely to be common may appear as rare or very rare in the list of species presented above, and the number of rare or very rare species may be overestimated in Fig. 5b. *Austronemura chilena*, *A. encoensis*, and *Notoperla archiplatae*, which appear to be very rare species, have widespread genera represented in the sampling primarily by immature specimens.

RESULTS

Species identified and distribution analysis

A total of 27 Patagonian stonefly species in six families were identified in both national parks (Table II), in addition to 8 taxa that could not be identified at the species level (see section *Challenges posed by immature specimens*). Twenty species were identified for NHNP and 19 for LANP. Eight species were exclusive to NHNP and seven to LANP (see Table II).

We generated 31 polygons for NHNP and 9 for LANP, with richness values ranging from 19 to 1 species per polygon (Fig. 3), and a median of 4.5 spp. for NHNP and 4 spp. for LANP. The highest richness was found in one polygon at LANP (n = 19), followed by one polygon at NHNP (n = 18). The analyses revealed an uneven distribution of species richness in both national parks; among the 31 studied polygons, seven exhibited the presence of 8 to 19 species for RN Province; five of these are within NHNP and two within LANP.

Within NHNP, five polygons with 8 to 19 species were identified, four located in the western half of the park (west of -71.4653) within the Northern Moist Forests ecosystem complex. The remaining polygon, with 15 species, lies within the Ecotone Steppe-Forest ecosystem complex in the southeast of NHNP (Fig. 3). The ecosystem complex with the lowest species richness in NHNP was Foothills (Patagonian Forest), with all polygons containing five or fewer species.

Within LANP, two polygons with 8 to 19 species were identified and are located in the eastern half of the park (Fig. 3), within the ecosystem complex of Northern Moist Forests and Transitional Cypress-Beech Forests. The remaining polygon, with seven species, is also within the Northern Moist Forests ecosystem complex in the northeast of LANP (Fig. 3). The Northern High-Andean ecosystem complex in LANP has no records of species.

In Figures 4a and 4b, the number of genera and species are presented for each ecoregion and ecosystem complex. In Figures 5a and 5b, the status of genera and species (e.g. rare, common) is presented for each ecoregion and ecosystem complex.

The ecosystem complexes of Northern Moist Forests, Transitional Cypress-Beech Forests, and Ecotone Steppe-Forest showed the highest richness in both genera (20, 17, and 15, respectively) and species (19, 16, and 14, respectively) (Figs. 4a and 4b, respectively); the lowest values for both genera and species were found in Foothills ecosystem complex.

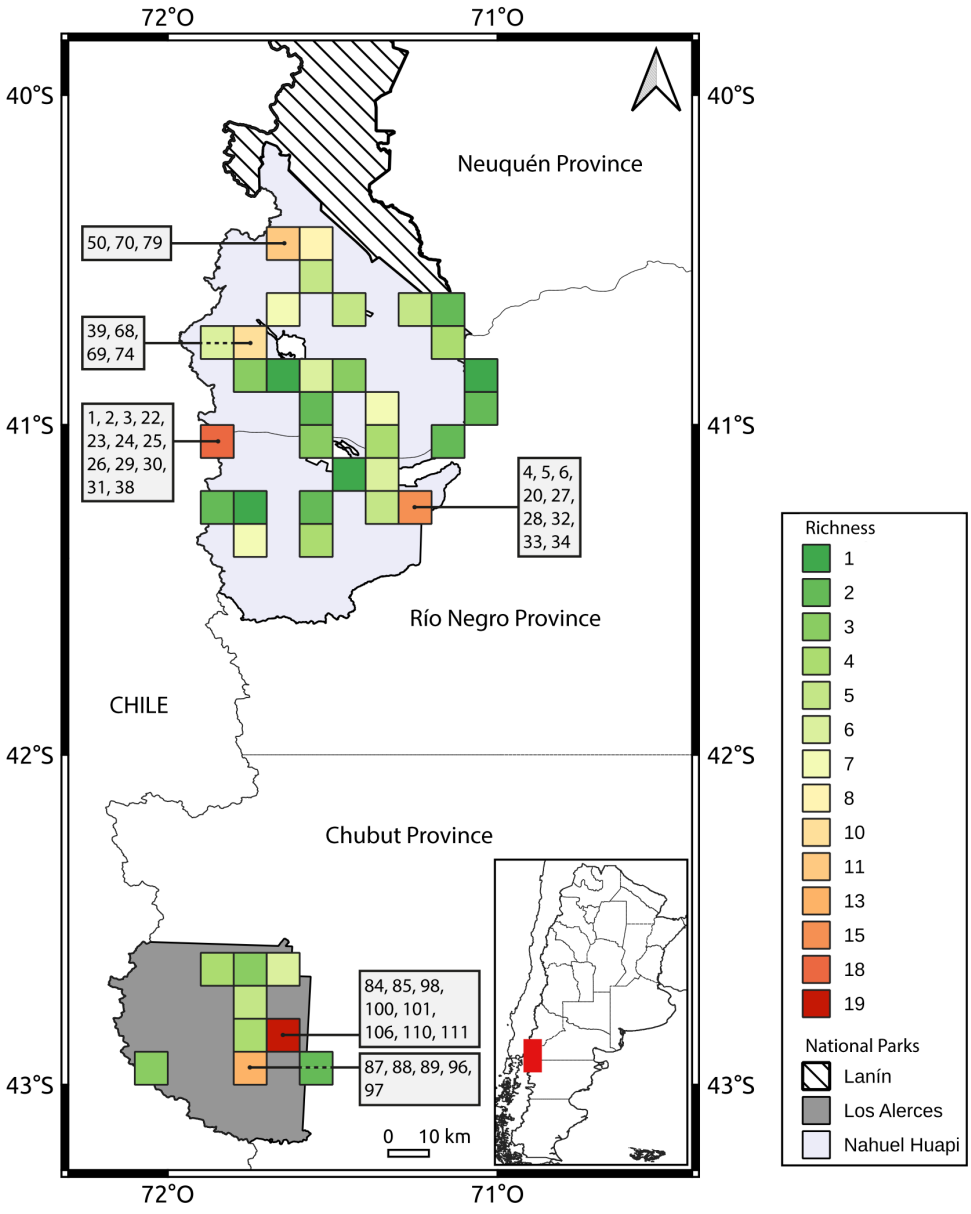

Figure 3. Polygons representing stonefly richness for Nahuel Huapi and Los Alerces National Parks.

Regarding the rarity status (Fig. 5), the Northern Moist Forests and the Transitional Cypress-Beech Forests ecosystem complexes showed the highest richness of very rare species (9 in each complex). The Northern Moist Forests (4) and Ecotone Steppe-Forests (3) had a higher concentration of rare species. The Northern Moist Forests and the Transitional Cypress-Beech Forests also had the highest richness of very rare (5 and 6, respectively) and rare genera (7 and 3, respectively). The Northern Moist Forests also had the highest richness of ubiquitous genera (8), followed by the Transitional Cypress-Beech Forests and the Ecotone Steppe-Forests (7 in each complex). In contrast, the Foothills ecosystem complex showed no rare or very rare genera or species, and only five genera and four species from the ubiquitous category, while

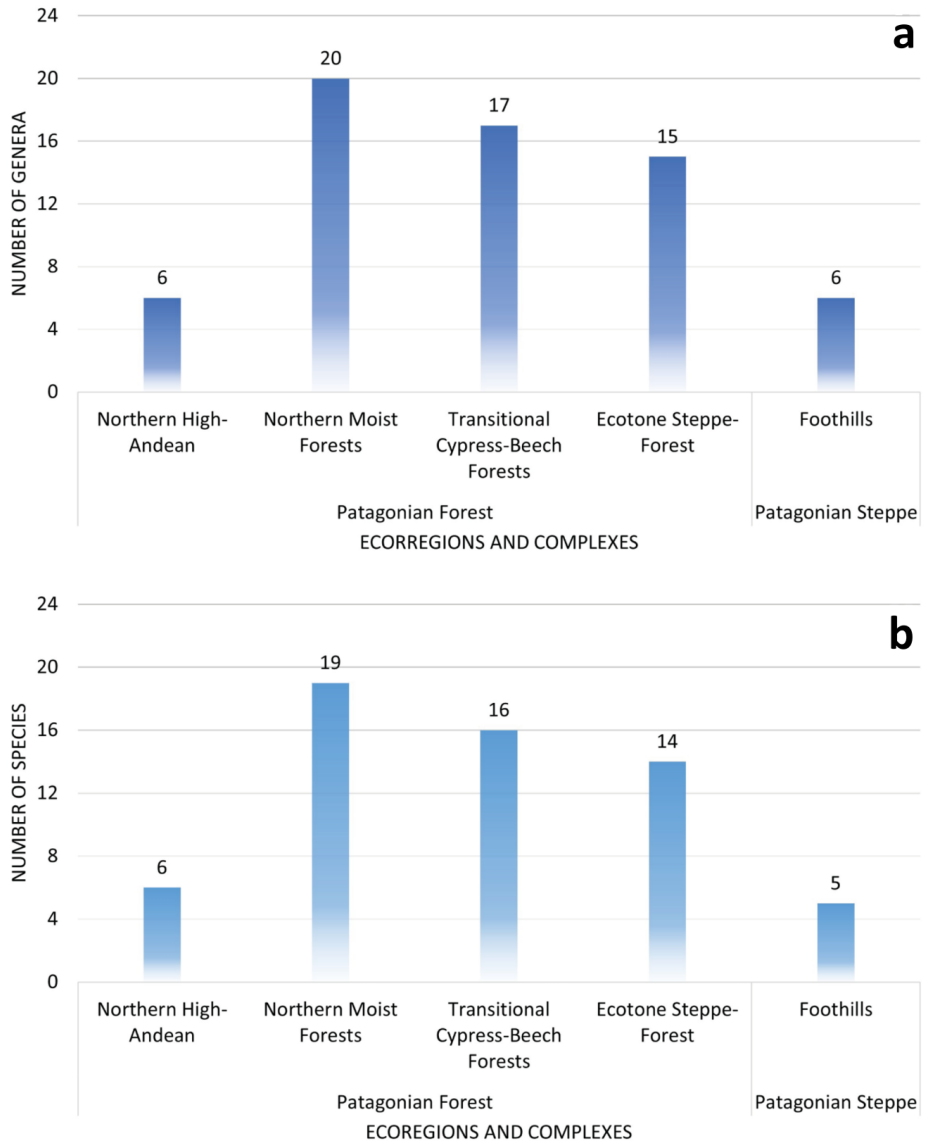

Figure 4. Total number of genera (a) and species (b) for each ecoregion and ecosystem complex.

the Northern High-Andean ecosystem complex showed low numbers in each category (between 3 and 0).

Cluster analysis among ecosystem complexes

According to the results of the cluster analysis (Fig. 6), the Northern High-Andean ecosystem complex clusters separately from the other ecosystem complexes at a higher dissimilarity level of 75% (similarity of 25%). The Transitional Cypress-Beech Forest and Foothills follow with dissimilarities of 66% (similarity of 34%) and 61.6% (similarity of 38.4%), respectively. The Northern Moist Forests of LANP showed a relatively moderate dissimilarity of 54.4% (similarity of 45.6%), while the Northern Moist Forests of NHNP and Ecotone Steppe-Forest cluster more closely, indicating a faunal dissimilarity of 47.4% (52.6% of similarity; see Supplementary Material - Table SI). The dissimilarity between the Northern Moist Forest of LANP and Transitional Cypress-Beech Forests ecosystem complex in the same national park is 73.16% (36.84% of similarity; see Table SI), while the Northern Moist Forest of NHNP shows

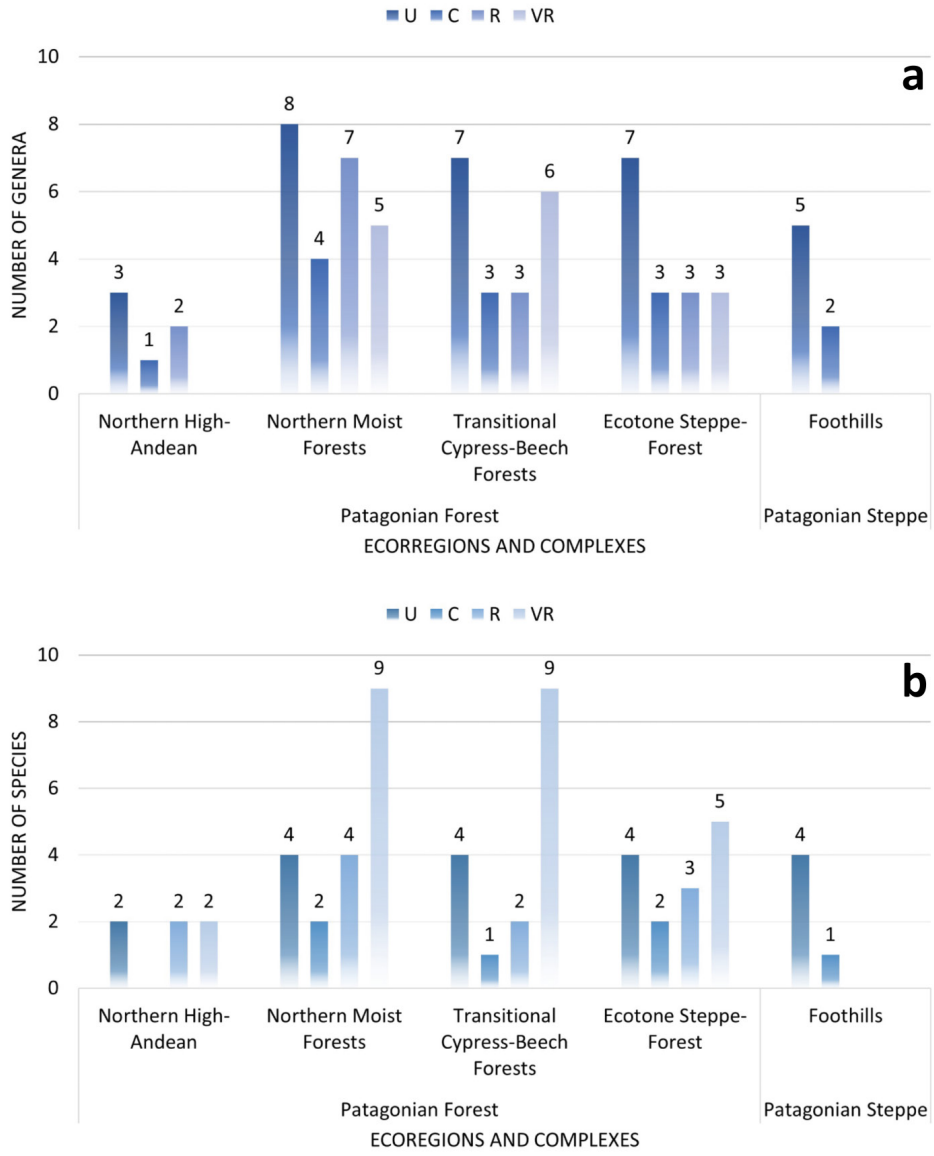

Figure 5. The current status of the genera (a) and species (b) in each ecoregion and ecosystem complex. Abbreviations: U, ubiquitous; C, common; R, rare; VR, very rare.

a dissimilarity of 50% with the same ecosystem complex in LANP (see Table SI). The CCC for the dendrogram was 0.85, indicating a strong fit between the dendrogram and the original Jaccard dissimilarity index, suggesting that the clustering faithfully represents the dissimilarity patterns between the ecosystem complexes.

The Mantel test revealed a weak negative correlation between Plecoptera species composition dissimilarity and geographic distance ($r = -0.166$; $p = 0.823$, based on 999 permutations). As the p-value exceeded the conventional significance threshold ($p > 0.05$), this suggests that geographic distance does not significantly explain the variation in species composition among the ecosystem complexes.

A total of 27 species were observed across the studied region. The species richness estimators Jack1, Jack2, and Chao2 reveal a significant gap in our understanding of stoneflies in the analyzed parks. Jack1 provides the most conservative estimate, indicating that 38 species may exist in addition to those currently documented. In contrast, Jack2 and

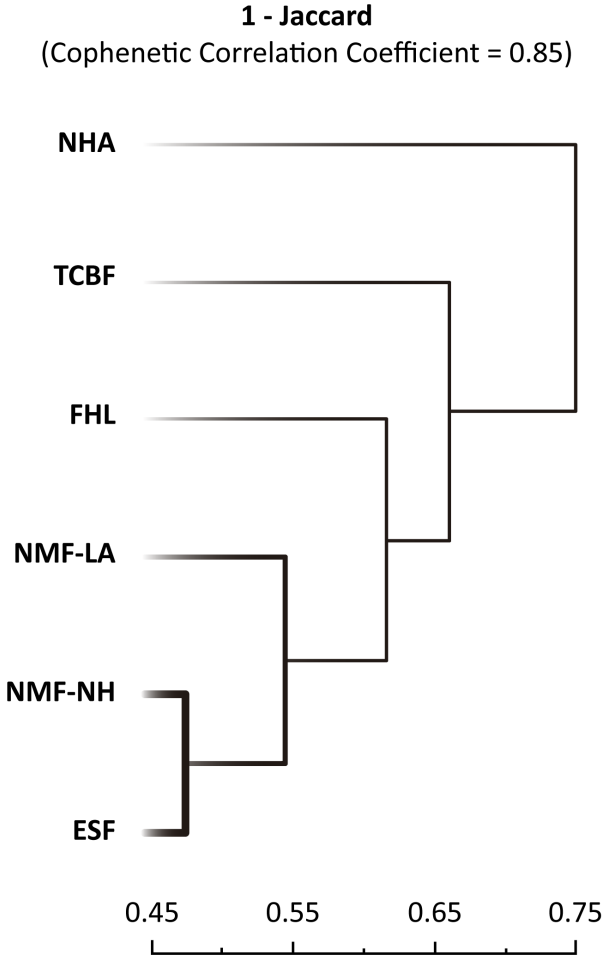

Figure 6. Cluster analysis based on faunal Jaccard dissimilarity between ecosystem complexes within LANP and NHNP. The cophenetic correlation coefficient (CCC) of 0.85 indicates a strong fit of the dendrogram to the original Jaccard dissimilarity index. Abbreviations: ESF, Ecotone Steppe-Forest; FHL, Foothills; NHA, Northern High-Andean; NMF-LA, Northern Moist Forests of LANP; NMF-NH, Northern Moist Forests of NHNP; TCBF, Transitional Cypress-Beech Forest.

Chao2 provide higher estimates, suggesting 45 and 50 species, respectively, which could imply 18 to 23 additional species that have yet to be documented.

New records and distribution novelties

We identified new country records (see Table I) for three species in Argentina (*Austronemoura encoensis*, *Chilenoperla puelche*, and *Teutoperla brundini*) (Tables I, II, and list of species below). New sites for RN Province were identified for two genera (*Neopentura semifusca* and *Notoperlopsis femina*; Table II and list of species below) and two species (*Chilenoperla elongata* and *Notoperla fasciata*; Table II and list of species below) within NHNP. In addition, new sites were identified for four genera within LANP (*Diamphipnopsis virescentipennis*, *Neuroperla schedingi*, *Alfonsoperla flinti*, and *Uncicauda testacea*).

Moreover, we identified new sites that represent the southernmost distribution for seven genera and one species (in LANP: *Neuroperla schedingi*, *Diamphipnoa helgae*, *Diamphipnopsis virescentipennis*, *Penturoperla barbata*, *Ericiataperla puerilis*, and *Teutoperla brundini*; in NHNP: *Neopentura semifusca* and *Notoperla archiplatae*; see Table II and list of species below); and one distribution expansion that represents a new site of northernmost distribution for one species (in NHNP: *Chilenoperla elongata*).

Based on the list provided by Pessacq & Duarte (2023), we have updated the number of species for Argentine Patagonia to 49 species in 33 genera. The following is a list of Plecoptera species from the NHNP and LANP, along with comments. Table II shows a detailed distribution. For each species, we provide information on the provinces where the species is recorded, the known stage and sex (M: male, F: female, L: larva), and indicate if it is a new record for the province (*). For additional information on provincial records, refer to Pessacq & Miserendino (2008) and Pessacq & Duarte (2023).

Abreviations: *Argentine provinces*: CH, Chubut; NQ, Neuquén; RN, Río Negro; SC, Santa Cruz; TF, Tierra del Fuego.

Austroperlidae. The family is generally distributed south of 35°S in South America (McLellan 2001). In Argentina, it is represented by three genera and three species (Pessacq &

Duarte 2023), with these species inhabiting all kinds of streams and rivers.

Andesobius barilochensis (Illies 1960). CH, NQ, RN. (M, F, L)

Notes: Very rare species. The species was described from the Ñireco stream near Bariloche (NHNP, RN), but it has not been found in decades and is likely locally extinct. McLellan (2001) states that the species is found in CH, NQ, and RN, but it has only been collected in northern NQ (P Pessacq, pers. obs.). This record was obtained from the literature and was not included in our analysis.

Klapopteryx kuscheli Illies 1960. CH, NQ, RN, SC. (M, F, L)

Notes: Ubiquitous species. It is the most widespread stonefly in the Patagonian Forest.

Penturoperla barbata Illies 1960. CH, NQ, RN. (M, F, L)

Notes: Very rare species. The record in LANP is the southernmost in Argentina.

Diamphipnoidae. The family comprises two genera and nine extant species endemics to the Patagonian Forest, from 35° S to 51° S (Pessacq et al. 2019). Two species are recorded in Argentina (Pessacq & Duarte 2023). These species inhabit low-order streams that are typically free of salmonids (P Pessacq, pers. obs.).

Diamphipnoa sp.

Notes: Rare larvae. The identification is based on early instar larvae.

Diamphipnoa helgae Illies 1960. CH, RN. (M, L).

Notes: Very rare species. The record in LANP is the southernmost in Argentina. The species was found in abundance at the park's only collection point. However, after a large fire in the area in 2017, the population declined. The RN record is based on literature (Illies 1960).

Diamphipnopsis virescentipennis (Blanchard 1851). CH, NQ, RN. (M, F, L).

Notes: Rare species. The species is found in low-order streams and has a patchy distribution throughout Argentine Patagonia. The records in LANP are the first for the national park and the southernmost in Argentina.

Eustheniidae. In Argentina, the family is represented by only one species, *Neuroperla schedingi* (Navás 1930) (Pessacq & Duarte 2023), while Chile has another more monotypic genus (*Neuroperlopsis patris* Illies). The family inhabits the south of 41° S, below 1,000 m. a.s.l. (Stark et al. 2009), in low-order streams that are typically free of salmonids (P Pessacq, pers. obs.).

Neuroperla schedingi (Navás 1930). CH, NQ, RN. (M, F, L).

Notes: Rare species. The species is recorded in NQ, RN, and CH. This is the first record in LANP and the southernmost for the family in Argentina.

Gripopterygidae. The family is represented by 25 genera and 40 species in Argentina, with 21 genera and 31 species recorded in the Argentine Patagonian region (Pessacq & Duarte 2023). The family inhabits the Patagonian Forest and the Steppe, in all kinds of streams and rivers.

Alfonsoperla flinti McLellan & Zwick 2007. CH. (M, F, L)

Notes: Very rare species. This is the first record for LANP.

Antarctoperla michaelseni (Klapálek 1904). CH, NQ, RN, SC, TF. (M, F, L)

Notes: Ubiquitous species. This species is one of the most widespread stoneflies in the Patagonian Forest, living in a variety of streams and rivers.

Aubertoperla illiesi (Froehlich 1960). CH, NQ, RN, SC. (M, F, L)

Notes: Ubiquitous species. This species is one of the most widespread stoneflies in the Patagonian Forest, living in a variety of streams and rivers.

Ceratoperla fazi (Navás 1934). CH, NQ, RN. (M, F, L)

Notes: Very rare species. This species has a patchy distribution throughout Argentine Patagonia.

Chilenoperla elongata Vera 2008. CH, RN*. (M, F, L)

Notes: Very rare species. This species has an apparently patchy distribution throughout Patagonian Forest, possibly due to its immature stages being indistinguishable from those of other *Chilenoperla* species and *Pelurgoperla personata*. The record in NHNP is new for RN.

Chilenoperla illiesi Nelson 1973. RN. (M, F)

Notes: Very rare species. This species is only known from the original description (Nelson 1973), and it has been recorded in RN, Bariloche. This record was obtained from the literature and was not included in our analysis.

Chilenoperla puelche Vera 2012. RN. (M, F, L)

Notes: Very rare species. The record of this species in NHNP is the first one for the country.

Chilenoperla sp./*Pelurgoperla personata* Illies 1963. CH, NQ, RN.

Notes: Species of *Chilenoperla* and *Pelurgoperla personata* cannot be differentiated based on immature stages (Vera 2008, 2012), which are the most frequently collected stage.

Ericiataperla puerilis (Illies 1963). CH. (M, F, L)

Notes: Very rare species. The record of this species in LANP is the southernmost in Argentina.

Limnoperla jaffueli (Navás 1928). CH, NQ, RN, SC. (M, F, L)

Notes: Ubiquitous species. This species is one of the most widespread stoneflies in the Patagonian Forest, found in a variety of streams and rivers.

Neopentura semifusca Illies 1965. NQ, RN*. (M, F, L)

Notes: Very rare species. The record of this species in NHNP is the first for RN and the southernmost in Argentina.

Notoperla archiplatae (Illies 1958). RN. (M, F, L)

Notes: Very rare species. The record in NHNP is the southernmost for the species.

Notoperla archiplatae/*N. magnaspina*. CH, NQ, RN, SC, TF.

Notes: Ubiquitous larvae. *Notoperla archiplatae* and *N. magnaspina* cannot be identified based on immature stages, which are the most frequently collected stage (Duarte, pers. obs.).

Notoperla fasciata McLellan 2006. CH, RN*. (M, F, L)

Notes: Very rare species. The record of this species in NHNP is the first for RN.

Notoperlopsis femina Illies 1963. CH, RN*. (M, F, L)

Notes: Very rare species. The record of this species in NHNP is the first for RN.

Rhithroperla rossi (Froehlich 1960). CH, NQ, RN. (M, F, L)

Notes: Common species in NHNP, it has only been recorded at one site in LANP.

Senzilloides panguipullii (Navás 1928). CH, NQ, RN. (M, F, L)

Notes: Rare species.

Teutoperla brundini Illies 1963. CH, RN. (M, F)

Notes: Very rare species. This is the first record of the species in Argentina. The record in LANP is the southernmost in the country.

Uncicauda testacea (Vera 2006). CH. (M, F, L)

Notes: Very rare species. This is the first record in LANP.

Notonemouridae. The family comprises four genera and 17 species found in the Patagonian Forest; all genera are present in Argentina, represented by nine species (Pessacq & Duarte 2023). In Argentine Patagonia, the family inhabits a variety of streams and rivers.

Austronemoura sp.

Notes: Common larvae, present in the Patagonian Forest. The different species cannot be identified based on immature stages, the most frequently collected stage.

Austronemoura chilena Aubert 1960. CH, RN. (M, F)

Notes: Based on adult records, this is a very rare species, but species of the genus *Austronemoura* are indistinguishable based on immatures.

Austronemoura encoensis Aubert 1960. RN. (M, F)

Notes: Based on adult records, this is a very rare species, but see above for species identification based on immatures. A new record for the country.

Neonemura barrosi Navás 1919. CH, RN. (M, F, L)

Notes: Rare species.

Neofulla sp.

Notes: Very rare larvae. Not all the different species can be identified based on immature stages, which are the most frequently collected stage.

Neofulla biloba (Aubert 1960). CH. (M, L)

Notes: Very rare species.

Udamocercia sp.

Notes: Ubiquitous larvae, present in the Patagonian Forest. The different species cannot be identified based on immature stages, which are the most frequently collected stage.

Udamocercia frantzi Illies, 1961. CH. (M, F)

Notes: Based on adult records, this is a very rare species, but species of the genus *Austronemoura* are indistinguishable based on immatures.

Perlidae. This is a cosmopolitan family. Currently, 29 species are recorded in Argentina, three of which are present in Patagonia (Pessacq & Duarte 2023). In this region, the family inhabits the Patagonian Forest, typically found in high-order rivers (P Pessacq, pers. obs.).

Pictetoperla gayi (Pictet 1841). CH, NQ, RN. (M, F, L).

Notes: Very rare species.

Pictetoperla repanda (Banks 1920) RN. (M, F, L).

Notes: Very rare species, known from Puerto Blest only (Illies 1964). This record was obtained from the literature and was not included in our analysis.

Pictetoperla sp.

Notes: Identification based on early instar larvae.

DISCUSSION

This contribution updates the number of Plecoptera species in two national parks in Patagonia, NHNP and LANP, to a total of 27 species. This represents more than half of the species known to inhabit the whole Patagonian region. We barely found differences in the total number of species identified between NHNP and LANP, despite NHNP being larger and more accessible. NHNP had 20 species, while LANP had 19, only a one-species difference between the parks. These findings contrast with Palma & Figueroa (2008), who reported higher species richness in Chile between latitudes -40° and -41° (35 and 34 species, respectively), which corresponds to NHNP, and a decrease in species richness at latitudes -43° and -44° (2 and 16 species, respectively), which aligns with LANP.

The number of species recorded on either side of the Andes showed notable differences. Palma & Figueroa (2008) reported 34 and 35 species in Chile at approximately the same latitude of NHNP, whereas our study recorded 26 taxa in NHNP, including taxa not identified at the species level. Pessacq et al. (2019) listed 45 species for Argentine Patagonia (east of the

Andes) and 78 for Chilean Patagonia (west of the Andes), indicating greater diversity on the Chilean side.

While we did not observe a decline in species numbers from north to south in our study area, it suggesting that the significant difference in species numbers between LANP and the latitudes cited by Palma & Figueroa (2008) is likely due to sampling bias. As suggested by the aforementioned authors, fewer collections have been conducted at those latitudes in Chile.

NHNP benefits from a network of roads and paths that facilitate access, whereas the western part of LANP has limited land access. NHNP is also 2.8 times larger than LANP; the aforementioned is reflected in the number of sites sampled in each national park (82 in NHNP and 29 in LANP), but the size, accessibility, and number of sites sampled differences did not result in a significant difference in the number of species or genera recorded in either park. Additionally, four polygons with more than 10 species were recorded in NHNP and two in LANP, a reasonable number given the difference in size of both national parks.

Our richness analysis identified different areas with the highest species richness in three different ecosystem complexes: Puerto Blest in the Northern Moist Forests, Cerro Chalhuaco in the Ecotone Steppe-Forest, and Futalaufquen Lake in the Transitional Cypress-Beech Forests. These areas also contained a high number of rare (4) and very rare (14) species (Table II). These high-richness areas in NHNP are subject to significant tourism, while LANP has less human activity, with only a few campsites near streams within the areas with the highest species richness.

The Northern Moist Forests and Ecotone Steppe-Forest complexes within NHNP shared 10 species and had the highest similarity in the cluster analysis (52.6%) (see Table SI). The Northern Moist Forests ecosystem complex found in both national parks shared nine species and showed a 50% similarity in the cluster analysis. In comparison, the Northern Moist Forests of LANP and the Ecotone Steppe-Forest of NHNP had a 41% similarity.

The most notable result from the cluster analysis is the higher similarity between the Northern Moist Forest of LANP and the same ecosystem complex in NHNP compared to its neighboring Transitional Cypress-Beech Forests ecosystem complex in LANP (36.84%). This finding suggests that ecosystem complexes, which reflect a range of ecological and environmental variables—such as humidity, temperature, vegetation type, and water flow dynamics—play a crucial role in shaping the distribution and association of stonefly species. The strong similarity between the Northern Moist Forests in both parks highlights the importance of shared environmental factors over geographic proximity in determining species composition. The results of the Mantel test ($r = -0.166$; $p = 0.823$, based on 999 permutations) suggest that geographic distance does not significantly explain the variation in species composition among the ecosystem complexes.

Despite the Northern Moist Forests being larger and better sampled, the Ecotone Steppe-Forest and Transitional Cypress-Beech Forests had comparable levels of genera and species richness (Figs. 4a and 4b). The Northern High-Andean and Foothills ecosystems, on the other hand, had fewer genera and species. Six genera were associated with specific ecosystem complexes: *Alfonsoperla*, *Ericiataperla*, and *Uncicauda* in the Transitional Cypress-Beech Forests of LANP; *Neopentura* and *Pictetoperla* in the Northern Moist Forests (with *Pictetoperla* present in both parks); and *Notoperlopsis* in the Ecotone Steppe-Forest of NHNP. Additionally, three Gripopterygidae genera (*Antarctoperla*,

Aubertoperla, and *Limnoperla*) were found across all ecosystem complexes except the Northern High-Andean. Five species were exclusively associated with specific ecosystems: *Neopentura semifusca* in the Northern Moist Forests; *Notoperlopsis femina* in the Ecotone Steppe-Forest; and *Alfonsoperla flinti*, *Ericiataperla puerilis*, and *Uncicauda testacea* in the Transitional Cypress-Beech Forests.

The number of rare and very rare taxa was comparably high in the Northern Moist Forests, Ecotone Steppe-Forest, and Transitional Cypress-Beech Forests ecosystem complexes (Figs. 5a and 5b). Both species richness and the number of rare and very rare species were greater in the Northern Moist Forests (Figs. 4 and 5), where most polygons with the highest species richness were also located (Fig. 3). In contrast, the Northern High-Andean and Foothills ecosystem complex had fewer species, genera, and rare taxa. The probable reasons for this greater observed species richness are several and include a higher density of sampled sites or more frequent visits, as well as the intrinsic higher diversity of the sampled region.

Notably, LANP marks the southernmost distribution limit for several genera, including *Neuroperla*, *Diamphipnoa*, *Diamphipnopsis*, *Penturoperla*, *Ericiataperla*, and *Teutoperla*. Most of these genera, along with their species, are rare or very rare (see Table II and species list above), and the extensive sampling and species identification across Patagonia confirm their bona fide distributional limits. Three newly documented species for Argentina, previously known only from Chilean Patagonia, were recorded in this study: *Austronemoura encoensis*, *Chilenoperla puelche*, and *Teutoperla brundini* (Froehlich 2010, Vera 2012). These species were classified as rare or very rare. Additionally, we identified new sites for the genera *Neopentura* and *Notoperlopsis* in NHNP, and *Diamphipnopsis*, *Neuroperla*, *Alfonsoperla*, and *Uncicauda* in LANP. These findings, along with new records for *Chilenoperla elongata* and *Notoperla fasciata* in NHNP, indicate that there is still much to learn about the distribution of stonefly species in Argentine Patagonia. For instance, species richness estimators suggest that we have only documented 54% to 71% of the actual species diversity, underscoring the need for continued collection efforts in unexplored areas.

Another critical issue highlighted by our study is the lack of knowledge about the life stages of many species, the so-called Haeckelian shortfall in biodiversity (Faria et al. 2020). Immature stages of several species remain indistinguishable, and many larval stages are still unknown. Paradoxically, larval stages are by far the most frequent finding in fieldwork and in collections because adulthood is relatively short and there are fewer opportunities to collect them. New approaches to studying those species will help us understand their distribution and status. This knowledge gap and stage bias have hindered the development of broader studies with significant relevance in the context of systematic research, as well as in applied studies related to ecological assessments, biomonitoring, and biogeographical or distributional studies.

Our study provides detailed data on insect distribution within two large national parks in northwestern Argentine Patagonia, identifying areas of high diversity that may be vulnerable to land use changes. Given the crucial role of insects in these ecosystems (Hynes 1976, Stewart & Stark 2002, Stark et al. 2009, Cheney et al. 2019, Sánchez-Bayo & Wyckhuys 2019, Frakes et al. 2021), their protection is essential. Accurate information represents a crucial first step in this process. The data presented here could have significant implications for conservation policies related to aquatic insects and will be

shared with the National Parks Administration in Argentina (LANP, NHNP).

A fundamental observation is that rare and very rare species do not occur outside of these national parks or in other protected Patagonian areas. Only *Alfonsoperla flinti*, *Neofulla biloba*, and *Ceratoperla fazi* were recorded in the Corcovado River basin in Chubut Province (Pessacq & Miserendino 2008). This emphasizes the critical role of these parks in preserving biodiversity. Increasing human activities in Patagonia, especially in aquatic environments, call for a more holistic conservation approach that includes aquatic insects as essential indicators of biodiversity.

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SUPPLEMENTARY MATERIAL

Table SI.

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TÁCIO DUARTE
https://orcid.org/0000-0002-4150-3857

GABRIEL M. MARTIN
https://orcid.org/0000-0003-2909-0444

DANIELLE ANJOS-SANTOS
https://orcid.org/0000-0002-2889-5964

PABLO PESSACQ
https://orcid.org/0000-0003-3143-8876

Centro de Investigación Esquel de Montaña y Estepa Patagónica (CIEMEP), Roca 780, 9200 Esquel, Chubut, Argentina

Correspondence to: **Pablo Pessacq**
E-mail: pablopessacq@yahoo.com.ar

Author contributions

All authors contributed to the study conception and design. PP, DAS and TD performed species sampling and material preparation. All authors wrote the first draft of the manuscript and then commented on previous versions of the manuscript. GMM conducted the species richness analyses. All authors read and approved the final manuscript.