Revista Brasileira de Entomologia
http://dx.doi.org/10.1590/S0085-56262014000400002

Conservation of mayflies (Insecta, Ephemeroptera) in Espírito Santo, southeastern Brazil

Fabiana Criste Massariol[1,4], Elaine Della Giustina Soares[2,3] & Frederico Falcão Salles[2]

[1]Programa de Pós-graduação em Biodiversidade Tropical, Laboratório de Sistemática e Ecologia de Insetos, Universidade Federal do Espírito Santo, Rodovia BR-101 Norte Km 60, Bairro Litorâneo, 29932-540 São Mateus-ES, Brazil.
[2]Departamento de Ciências Agrárias e Biológicas, Centro Universitário Norte do Espírito Santo, Universidade Federal do Espírito Santo, Rodovia BR-101 Norte Km 60, Bairro Litorâneo, 29932-540 São Mateus-ES, Brazil. ffsalles@gmail.com
[3]Present address: Instituto Latino-Americano de Ciências da Vida e da Natureza, Universidade Federal da Integração Latino-Americana, Foz do Iguaçú, Paraná, Brazil. elainedgs@gmail.com
[4]Corresponding author: fcmassariol@gmail.com

ABSTRACT. Conservation of mayflies (Insecta, Ephemeroptera) in Espírito Santo, southeastern Brazil. Ephemeroptera exhibits great diversity among bodies of freshwater in the Atlantic Forest, a biome that is suffering from massive human impact. Within this context, the creation of conservation units using biological information is more recommended than economic, cultural, or political criteria. The distribution pattern of 76 Ephemeroptera species was analyzed using the biogeographical methods Parsimony Analysis of Endemicity and Network Analysis Method in order to infer relevant areas for conservation of the mayfly community in Espírito Santo. The results obtained from both analyses were largely congruent, and pointed out four relevant areas for conservation: two in the south of the state, where conservation units or priority areas for conservation are well established; and two in the north, a region in the state where little conservation efforts have been historically done. Therefore, based on our analyses on mayflies, we recommend the expansion of the existing APCs or the creation of new APCs on the north of Espírito Santo.

KEYWORDS. Atlantic Forest; biodiversity; distribution pattern; endemism; macroinvertebrates.

Conservation units are created by public managers and are of great importance to the *in situ* conservation of species, populations, and ecosystems. In addition to ensuring the preservation of biodiversity, conservation units are an important means of guaranteeing the quality and quantity of the water supply (Medeiros *et al.* 2011). Approximately 45% of threatened or endangered species depend on aquatic habitats and wetlands (Clark 1999). Therefore, to delimit and create a conservation unit, it is important to consider the hydrographical basin in which it is located (Clark 1999; Moulton & Souza 2006). Besides that, despite the above mentioned dependence on aquatic ecosystems, many studies have been conducted based on charismatics species (usually vertebrates), leading to a potentially problematic taxonomic bias in conservation (Clark & May 2002). In this sense, the use of macroinvertebrates in these kinds of studies is very important.

For the establishment of a conservation unit, economic, cultural, and political factors are more decisive than ecological principles, which are merely one among many criteria for the selection of a preservation site; moreover, there are several biological factors that should be considered (Soulé & Simberloff 1986). Accordingly, many conservation units have been created in Brazil and also in Espírito Santo without taking into consideration the most appropriate criteria.

The criterion of endemism is utilised to indicate a specific location as a priority for conservation (Carvalho 2004; Chen & Bi 2007; Löwenberg-Neto 2011). Although there are many concepts used to define areas of endemism, most authors agree that these areas have a significant number of exclusive species (Nelson & Platnick 1981). Indeed, areas of endemism are biogeographic elements that are used to indicate areas to be conserved because of unique features of biodiversity (Löwenberg-Neto 2011).

The Atlantic Forest is one of the most biodiverse and endangered ecosystems in the world, occupying the fourth position on the hottest hotspots in the world (Myers *et al.* 2000). It includes a large region that extends over the coastal mountain range along the Atlantic Ocean, in the northeast, southeast, and south regions of Brazil, also including eastern Paraguay and northern Argentina (IPEMA 2005). This biome that originally occupied approximately 90% of the State of Espírito Santo (southeastern Brazil) was reduced to only 9% after drastic environmental changes (IPEMA 2005; Passamani 2007). Fragmentation and habitat loss are the largest factors regarded as responsible for the loss of biodiversity and are the main processes contributing to landscape change (Fischer & Lindenmayer 2007).

Mayflies (Ephemeroptera) exhibit a high diversity and abundance in rivers of various sizes in the Atlantic Forest (Crisci-Bispo *et al.* 2007; Salles *et al.* 2010), and play an indispensable role in the food chain participating in the nutrient cycling, providing a food source for fish, birds, and other invertebrates (Waltz & Burian 2008). This order is the oldest group among winged insects (Britain & Sartori 2003) and have been widely used as bioindicators of water and ecological integrity (Baptista *et al.* 2007; Buss & Salles 2007), mainly because they exhibit significant variation in sensitivity to various contaminants including ammonia, nitrite, nitrate, metal, and other chemicals (Hickey & Clements 1998; Beketov 2004).

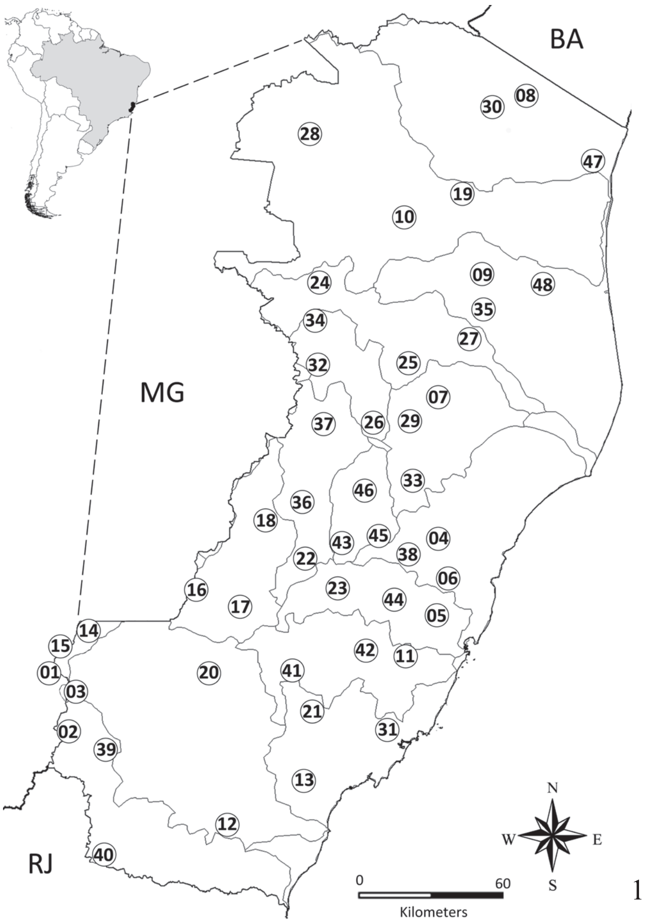
The aim of this paper, therefore, is to investigate relevant areas for conservation of the mayfly community in Espírito Santo, Brazil. Besides that, the aim is also to check if the existing conservation units or priority areas for conservation in the state encompass the relevant areas found in this work.

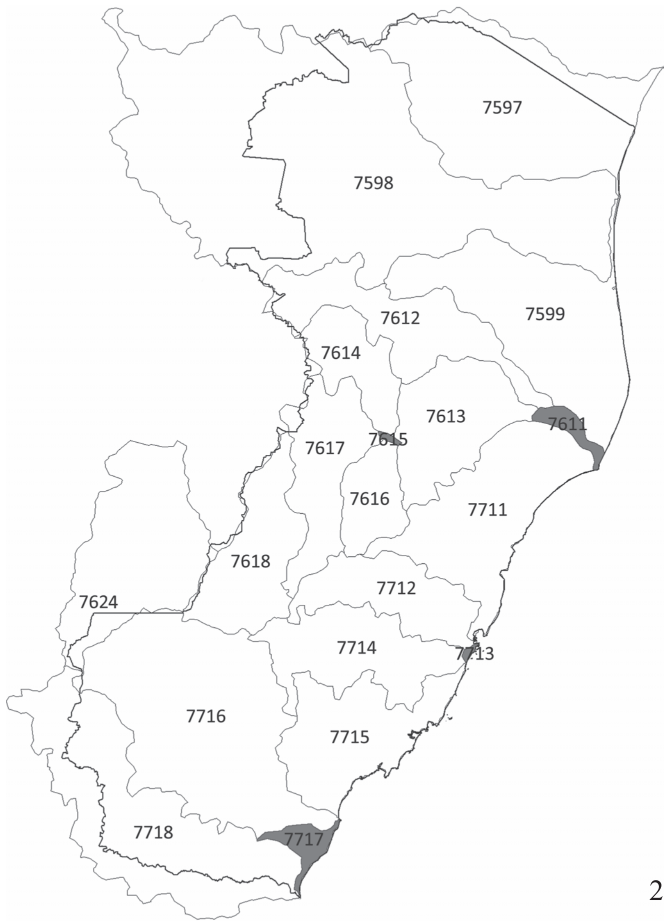
MATERIAL AND METHODS

Study area. Espírito Santo has an area of 45,597 km^2 and is located in southeastern Brazil, bounded by the states of Bahia, Minas Gerais, and Rio de Janeiro in the north, west, and south, respectively (Fig. 1). The topography is mountainous, with elevations ranging from sea level to 2897 m (IPEMA 2005). The state has 20 ottobasins, at level 04 (Fig. 2): 10 are basins (basins of the São Mateus, São José, Pancas, Santa Maria do Rio Doce, Guandu, José Pedro, Santa Maria da Vitória, Jucu, Itapemirim, and Itabapoana rivers), and 10 are interbasins (interbasins of the Itaúnas, Barra Seca, Norte, Bananal, Córrego do Ouro, Santa Joana, Piraquê-Açu, Aribiri, Benevente, and Córrego São Salvador rivers) (IJSN 2009). The state has 14 federal conservation units and 17 state conservation units, for a total of 31 conservation areas (Fig. 3) occupying 2.66% of the state area (119,559.8 ha). Most of the conservation units comprise less than 2,500 ha, and only four contain more than 10,000 ha: *Reserva Biológica de Sooretama*, *Parque Nacional do Caparaó*, *Parque Nacional dos Pontões Capixabas*, and *Parque Estadual Paulo César Vinha*. The first two units together represent the largest continuous forest remnants of the state and have great importance for the conservation of biodiversity (IPEMA 2005).

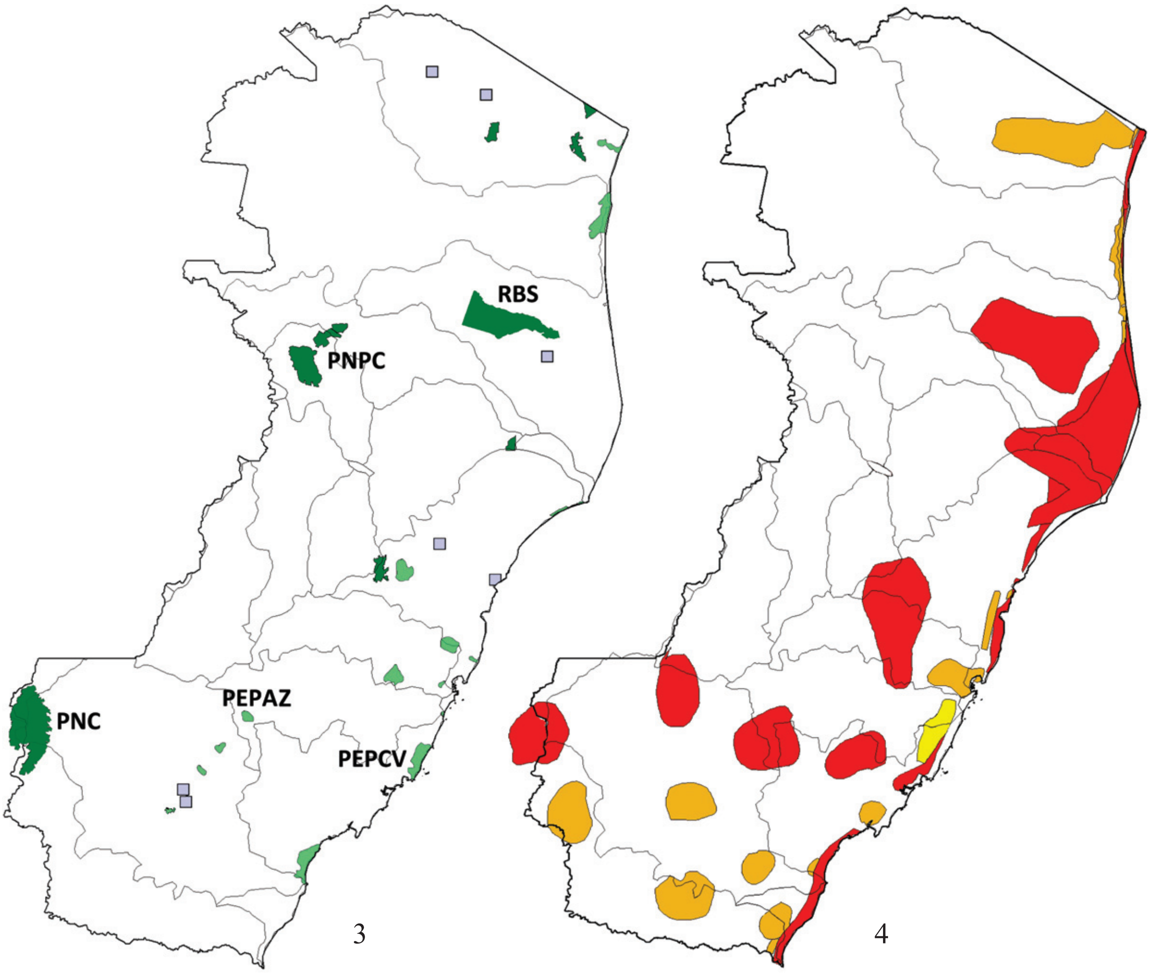
Projects sponsored by the *Ministério do Meio Ambiente* (MMA), part of the executive branch of the federal government, have indicated 182 priority areas for conservation (APCs) in Brazil (MMA 2000). Twenty-six APCs are in Espírito Santo (concentrated in the south): 12 of extreme biological importance, 13 of very high importance, and only one of high importance (Fig. 4).

Sampling and identification. We collected nymphs from 48 sampling sites between October 2011 and August 2012





Figs. 1–2. Map of Espírito Santo State. 1. Geographical position of Espírito Santo and the 48 collection points. 2. Hydrographic ottobasins (level 04) in Espírito Santo. Ottobasins in gray were not sampled (Modified from IJSN 2009).



Figs. 3–4. Map of conservation areas in Espírito Santo. 3. Federal, state, and private conservation units (in dark green, light green and light blue respectively). Private reserves are not represented according to their real limits. Acronyms of conservation units mentioned in this paper: RBS, *Reserva Biológica de Sooretama*. PNPC, *Parque Nacional Pontões Capixabas*. PNC, *Parque Nacional do Caparaó*. PEPAZ, *Parque Estadual de Pedra Azul*. PEPCV, *Parque Estadual Paulo César Vinha* (Modified from IPEMA 2005). 4. Priority areas for conservation according to MMA 2000. APCs in red with extremely high priority, in orange with very high priority, and in yellow with high priority.

(Fig. 1, Table I) in Espírito Santo and along its border with Minas Gerais. The sampling points were distributed in a standardised way—three in each ottobasin at different elevations and river widths. This standardisation was employed to obtain a more complete sampling, as these factors directly influence the composition of Ephemeroptera fauna (Domínguez & Valdez 1992; Baptista *et al.* 2001; Gallardo-Mayenco 2003). Only four ottobasins were not sampled (interbasins of the Norte, Aribiri, Córrego São Salvador, and Córrego do Ouro rivers) because they are influenced by seawater or have a reduced size (Fig. 2).

In Brazil, hydrographic basins are defined as operating territorial units for management of water resources, according to the law nr. 9433 (BRASIL 1988), and the classification in ottobasins has been used by *Conselho Nacional de Recursos Hídricos*. Therefore, the choice to work at ottobasins level was because this geographic information system is more appropriate to make decisions on water resources (ANA 2006). Besides, the rapid assessment approaches are enough to show the diversity profile of Ephemeroptera, due to the availability of the immature stages during most of the time (Edmunds *et al.* 1976) and the easy and inexpensive identification of specimens (Lenat & Barbour 1994; Resh 1994).

Collections were performed in 50-m stretches in rivers with small to medium widths and in 100-m stretches in large rivers. Ten samples in each section were collected with the aid of D-shaped net (aperture of 0.5 mm) in which a sweeping of the shadow area of net was performed. The sampling distribution in each stretch was made according to substrate availability, these included slabs, stone, gravel, sand, root, macrophytes, marginal vegetation, bottom litter, riffle litter. The samples were collected in this way to prevent the sampling effort from influencing the results.

All samples collected were fixed in 80% ethanol. The identifications were made based on Domínguez *et al.* (2006) and Salles (2006), assisted when necessary by articles relevant to each taxon. Morphotypes, *i.e. Paracloeodes* species 1, *Paracloeodes* species 2, were used for those specimens

Table I. Sample sites in the state of Espírito Santo and its border with Minas Gerais (Alto Caparaó and Espera Feliz), followed by the municipality, locality, geographic coordinates, and elevations.

Point	Collection date	Municipalities	Locality	Coordenates	Elevation (m)
PT 01	13/x/2011	Alto Caparaó	PARNA Caparaó, Vale Encantado	S 20°24'38.7" W 41°50'03.6"	1976
PT 02	14/x/2011	Espera Feliz	PARNA Caparaó, Pedra Menina	S 20°37'30.3" W 41°49'27.1"	884
PT 03	16/x/2011	Alto Caparaó	PARNA Caparaó, Rio São Domingos, Cachoeira da Farofa	S 20°28'19.5" W 41°49'41.7"	1972
PT 04	02/xi/2011	Ibiraçú	Cachoeirão	S 19°53'23.4" W 40°25'42.0"	54
PT 05	05/xi/2011	Serra	Distrito de Queimados, Rio Santa Maria	S 20°11'09.7" W 40°23'08.0"	0
PT 06	05/xi/2011	Serra	BR 101, Ponte do Bagaço	S 20°03'33.7" W 40°22'42.7"	9
PT 07	08/xi/2011	Linhares	Cachoeira de Angeli	S 19°20'59.1" W 40°25'17.5"	66
PT 08	16/xi/2011	Pedro Canário	Assentamento Castro Alves, Cachoeira da Mata	S 18°12'10.8" W 40°04'37.9"	35
PT 09	22/xi/2011	Jaguaré	Santa Maria, Cachoeira do Bereco	S 18°53'04.5" W 40°12'23.1"	45
PT 10	24/xi/2011	Nova Venécia	Santa Rita do Pip Nuk	S 18°39'51.4" W 40°30'44.9"	74
PT 11	02/i/2012	Viana	Rio Formate	S 20°20'0.90" W 40°30'52.4"	84
PT 12	10/i/2012	Atílio Vivacqua	Poço Dantas	S 20°59'12.2" W 41°11'08.1"	59
PT 13	11/i/2012	Rio Novo do Sul	Cachoeira Venezuela	S 20°48'39.3" W 40°53'48.1"	331
PT 14	17/i/2012	Iúna	Pouso Alto, Rio Pouso Alto	S 20°18'23.1" W 41°44'50.1"	329
PT 15	17/i/2012	Iúna	Bar Hidrolândia, Rio Brás	S 20°19'25.6" W 41°48'45.5"	804
PT 16	18/i/2012	Brejetuba	São Domingos, Fazenda Leogildo	S 20°05'13.8" W 41°19'54.1"	656
PT 17	18/i/2012	Afonso Cláudio	Cachoeira Santa Luzia	S 20°09'19.9" W 41°08'32.3"	457
PT 18	19/i/2012	Laranja da Terra	Cachoeira Criminosa	S 19°49'0.30" W 41°00'43.2"	232
PT 19	27/i/2012	São Mateus	Cachoeira Japira	S 18°34'39.1" W 40°16'58.0"	8
PT 20	30/i/2012	Conceição do Castelo	Rio Castelo, Cachoeira Bicame	S 20°24'07.5" W 41°16'06.5"	477
PT 21	31/i/2012	Alfredo Chaves	Cachoeira Águas de Pinon	S 20°32'58.9" W 40°51'18.8"	566
PT 22	01/ii/2012	Itarana	Barra Jatibocas, Rio Jatibocas	S 19°58'45.6" W 40°52'55.5"	483
PT 23	01/ii/2012	Santa Maria de Jetibá	Cachoeira Ilha Berger	S 20°03'27.5" W 40°46'25.0"	709
PT 24	07/ii/2012	Águia Branca	Brejão, Boa Vista, Cachoeira Luizana	S 18°55'36.2" W 40°50'25.2"	238
PT 25	02/iii/2012	Governador Lindemberg	Fazenda Sugo, Córrego	S 19°14'14.6" W 40°30'09.7"	147
PT 26	29/iii/2012	Colatina	Cachoeira do Oito	S 19°28'13.8" W 40°36'40.7"	70
PT 27	12/iv/2012	Sooretama	Rio São José	S 19°07'33.1" W 40°14'26.1"	24
PT 28	17/iv/2012	Ecoporanga	Cachoeira 2 de setembro	S 18°20'55.9" W 40°52'23.4"	366
PT 29	23/iv/2012	Marilândia	Córrego Limoeiro	S 19°26'10.5" W 40°31'34.7"	87
PT 30	27/iv/2012	Montanha	Fazenda Esplanada, Cachoeira Goela da Onça	S 18°14'42.3" W 40°10'43.8"	72
PT 31	04/v/2012	Guarapari	Pousada Rio das Pedras	S 20°37'05.9" W 40°34'55.4"	35
PT 32	14/v/2012	Pancas	Pratinha, Cachoeira do Bassini	S 19°14'19.6" W 40°50'49.7"	189
PT 33	15/v/2012	João Neiva	Acioli, Rio Ubás	S 19°41'09.5" W 40°28'37.2"	66
PT 34	24/v/2012	Pancas	Vila Verde, Rio Sumidouro do Pancas, Cachoeira do Gilles	S 19°03'02.5" W 40°52'27.9"	95
PT 35	12/vii/2012	Sooretama	REBIO Sooretama, Córrego Rodrigues	S 19°01'36.6" W 40°13'39.0"	44
PT 36	16/vii/2012	Itaguaçú	Sobreiro, Cachoeira Cristofari	S 19°45'20.9" W 40°55'45.6"	160
PT 37	17/vii/2012	Colatina	Itapina, São Pedro Frio, Córrego na estrada	S 19°27'06.6" W 40°48'54.4"	265
PT 38	30/vii/2012	Santa Teresa	Córrego na subida de Santa Teresa	S 19°56'13.2" W 40°28'44.1"	187
PT 39	31/vii/2012	Guaçuí	São Tiago, Cachoeira do Carlito	S 20°41'56.2" W 41°38'07.4"	550
PT 40	31/vii/2012	Bom Jesus do Norte	Ilha do Vicente, Rio Itabapoana	S 21°06'53.6" W 41°41'30.9"	64
PT 41	01/viii/2012	Domingos Martins	Cachoeira Floriano	S 20°24'41.5" W 40°56'34.2"	1044
PT 42	01/viii/2012	Domingos Martins	Estrada para Cascata do Galo, Rio Jucu Braço Norte	S 20°19'00.2" W 40°39'24.6"	464
PT 43	02/viii/2012	Santa Teresa	Cachoeira Zanotti	S 19°55'17.4" W 40°44'38.3"	448
PT 44	02/viii/2012	Santa Leopoldina	Rio que passa na estrada para Hospedaria Pau a Pique	S 20°07'36.5" W 40°33'03.6"	370
PT 45	03/viii/2012	Santa Teresa	Rio 5 de novembro, primeira ponte	S 19°52'48.8" W 40°36'38.6"	290
PT 46	03/viii/2012	São Roque do Canaã	Rio Santa Maria do Rio Doce, Cachoeira São Pedro	S 19°42'40.3" W 40°39'42.3"	69
PT 47	04/viii/2012	Conceição da Barra	Córrego Angelim	S 18°27'09.3" W 39°47'57.9"	1
PT 48	04/viii/2012	Jaguaré	Córrego em Água Limpa	S 18°55'40.4" W 39°59'09.9"	40

that did not fit into any species concept. They may represent species new to science or undescribed stages of previously described species. The specimens are deposited in the *Coleção Zoológica Norte Capixaba* (CZNC) of the *Universidade Federal do Espírito Santo* (UFES), in São Mateus.

Data analysis. Two methods of historical biogeographic were used to analyse the data: Parsimony Analysis of Endemicity (PAE) and Network Analysis Method (NAM), both can be used to delimitate areas of endemism. PAE (Morrone 2014) has also been used to indicate priority areas for conservation (*e.g.*, Caviers *et al.* 2002; Chen & Bi 2007; Huang *et al.* 2010) and NAM, recently developed by Dos Santos *et al.* (2008, 2012), to date has not been used for this propose. However, NAM has many advantages when compared to PAE, such as: 1) independence on predefined areas; 2) considers earth's curvature; 3) evaluates the randomness in data structure; and 4) has a high relative stability of results to scale change (Dos Santos *et al.* 2008).

Although the collections were conducted quantitatively, the data were analysed qualitatively. After specimen identifications, matrices of presence and absence were constructed for PAE, and a set of species and its respective geographical coordinates were constructed for NAM. The areas resulting from these analyses were not denominated areas of endemism because the species whose geographic distribution extends outside the Espírito Santo area were not excluded from the analyses, so only the distribution within the state was taken into consideration.

Parsimony Analysis of Endemicity. We performed two separate analyses using localities as the operational geographical units: PAE by collection point and PAE by ottobasin. The data matrix was generated in Microsoft® Office Excel 2010, and the taxa were coded as absent (0) or present (1). A hypothetical area coded with zeros was used to root the cladogram; thus, the endemic areas were grouped by the presence of taxa. The matrices were analysed with TNT (Goloboff *et al.* 2004) using traditional search with 500 replicates. A strict consensus cladogram of the resulting trees was obtained using Winclada 1.0 (Nixon 1999) with fast optimization. The collection points and ottobasins with two or more exclusive species in Espírito Santo were mapped using the programme DIVA-GIS 7.5.0 (Hijmans *et al.* 2005).

Network Analysis Method. The distribution patterns of species of Ephemeroptera were analysed through NAM based on sympatry inference (Dos Santos *et al.* 2008, 2012). The analysis was performed using the software R 2.14.2 (R Development Core Team 2011) and the packages SyNet (Dos Santos *et al.* 2012) and TKRplot (Tierney 2010).

The data were managed to estimate the minimum spanning tree for each species, and the orthodromic distances were calculated. From this result, two matrices of spatial association were inferred: the cost of spatial homogenisation (ACSH) and the topological resemblance (MST). The analysis involved only UCs and diads that satisfied both thresholds, and those that were absent from one of the matrices were discarded. The next step was to choose the cut-off value (maximum distance between the two points considered to be sympatric) used to calculate the basal network. The basal network was achieved from the reweighted topological resemblance > 0.886 and ACSH < 26.091 km. The binary matrix generated corresponded to the basal network to be analysed by NAM.

UCs are presents in a large network, and existing intermediate species are typically associated with these UCs. Thus, the removal of intermediate species segregates UCs and diads. The resulting cleavogram shows the spatial relationship among the species in a net context and represents a simplified technique to illustrate the division of groups with the removal of intermediate species. The spatial expression patterns of UCs and diads were mapped using the software DIVA-GIS 7.5.0 (Hijmans *et al.* 2005).

RESULTS

A total of 76 species (Appendix 1) were found in 48 sampling sites, for a total of 658 distribution records from Espírito Santo and its borders with Minas Gerais and Rio de Janeiro.

PAE by collection point. The analysis of the data matrix (Table II) produced 34 equally parsimonious trees with 432 steps, a consistency index of 17, and a retention index of 19. The strict consensus cladogram (Fig. 5) showed at the base of the cladogram the presence of six species widely distributed in the state: *Americabaetis alphus* Lugo-Ortiz & McCafferty, 1995; *Camelobaetidius billi* Thomas & Dominique, 2000; *Camelobaetidius rufiventris* Boldrini & Salles, 2009; *Paracloeodes eurybranchus* Lugo-Ortiz & McCafferty, 1996; *Paracloeodes waimiri* Nieto & Salles, 2006; and *Farrodes carioca* Dominguez, Molineri & Peters, 1996. Furthermore, some points or groups showed only one exclusive species: PT 10 (*Tricorythopsis rondoniensis* Dias, Cruz & Ferreira, 2009), PT 16 (*Lachlania* sp. 2), PT 21 (*Lachlania* sp. 1), PT 35 (*Adebrotus lugoi* Salles, 2010), PT 37 (*Baetodes liviae* Polegatto & Salles, 2008), PT 41 (*Paracloeodes* sp. 2), PT (26+19(10+27)) (*Traverella insolita* Nascimento & Salles, 2013), and PT (10+27) (*Harpagobaetis gulosus* Mol, 1986).

Four points (Fig. 6) with two or more exclusive species each resulted from the analyses: PT 02 defined by *Paracloeodes* sp. 1 and *Leptohyphodes inanis* (Pictet, 1843); PT 08 defined by *Callibaetoides caaigua* Cruz, Salles & Hamada, 2013 and *Miroculis* sp. 1; and PT 42 defined by *Spiritiops* sp. 1 and *Thraulodes* sp. 1. Sample site PT 27 showed the highest number of exclusive species, *Camelobaetidius juparana* Boldrini & Salles, 2012; *Tricorythopsis spongicola* Limas, Salles & Pinheiro, 2011; and *Oligoneuria amandae* Salles, Soares, Massariol & Faria, 2014, plus two: *Ha. gulosus* shared with PT 10 and *Ta. insolita* shared with PT 16 and 26.

PAE by ottobasin. The analysis of the data matrix (Table III) produced six equally parsimonious trees with 182 steps, a consistency index of 41, and a retention index of 42. The strict consensus cladogram (Fig. 7) showed ten ottobasins and groups of them (Figs. 8–9) with two or more exclusive species each. The resulting ottobasins exhibited a nested pattern, with some groups subordinate to others.

All the ottobasins analysed were grouped by eight species: *Am. alphus*; *Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996; *Cm. billi*; *P. eurybranchus*; *P. waimiri*; *Waltzoyphius fasciatus* Lugo-Ortiz & McCafferty, 1995; *Tricorythopsis minimus* (Allen, 1973); and *F. carioca*. The basins and interbasins of the Pancas (7614), São Mateus (7598), São José (7612), Guandu (7618), Santa Maria do Rio Doce (7616), Santa Joana (7617), Santa Maria da Vitória (7712), Piraquê-Açu (7711), Bevente (7715), Jucu (7714), Itabapoana (7718), José Pedro (7624), and Itapemirim rivers (7716) were grouped by five exclusive species: *Baetodes iuaquita* Salles & Nessimian, 2011; *Cm. rufiventris*; *Leptohyphes plaumanni* Allen, 196; *Tricorythopsis* sp. 1; and *Ta. insolita.* All the thirteen ottobasins mentioned above

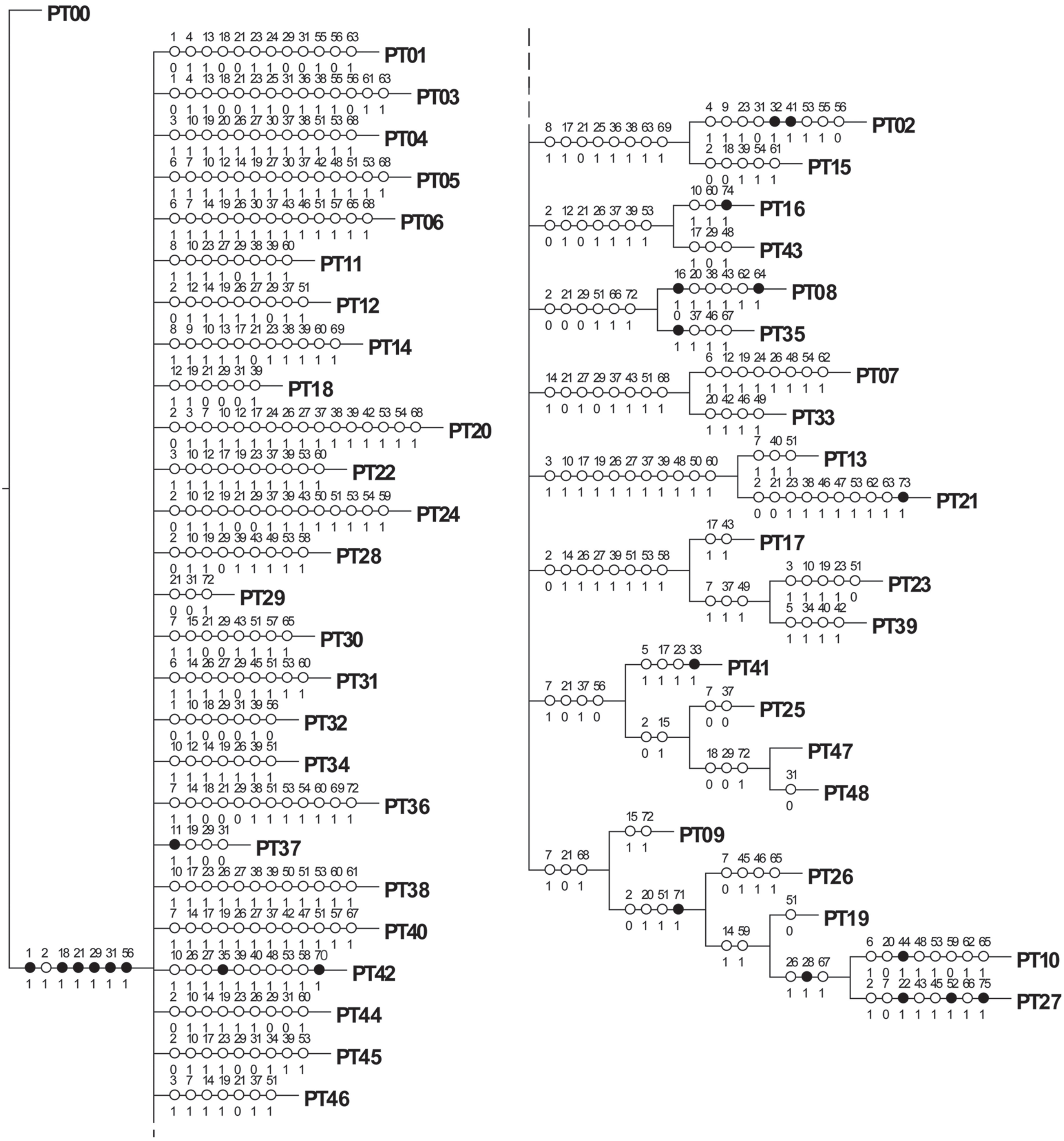


Fig. 5. Strict consensus cladogram (fast optimization) resulting from PAE by collection points. The white circles correspond to homoplasies, and the black circles denote species that occur exclusively in this area. The number above the circles represents species code; number below the circles means present (1) or absent (0).

jointly with the interbasin of the Bananal river (7613) were defined by three exclusive species: *Callibaetis* sp. 1; *Camelobaetidius francischettii* Salles, Andrade & Da-Silva, 2005; and *Cloeodes* sp. 1.

In the north of the state, the interbasin of the Itaúnas river (7597) showed *Cl. caaigua* and *Miroculis* sp. 1 as exclusive species. The basin of the São José river (7612) presented three exclusive taxa: *Cm. juparana*, *Ti. spongicola*, and *O. amandae*. These basins in conjunction with the basin of the São Mateus river (7598) were grouped by the presence of the exclusive species *Ha. gulosus* and *Hylister obliquus* Nascimento & Salles, 2013.

The resulting ottobasins in the south of the state had a little more complex hierarchical pattern. The basin of the

Fig. 6. Spatial expression of collection points resulting from PAE, superimposed on the ottobasin map.

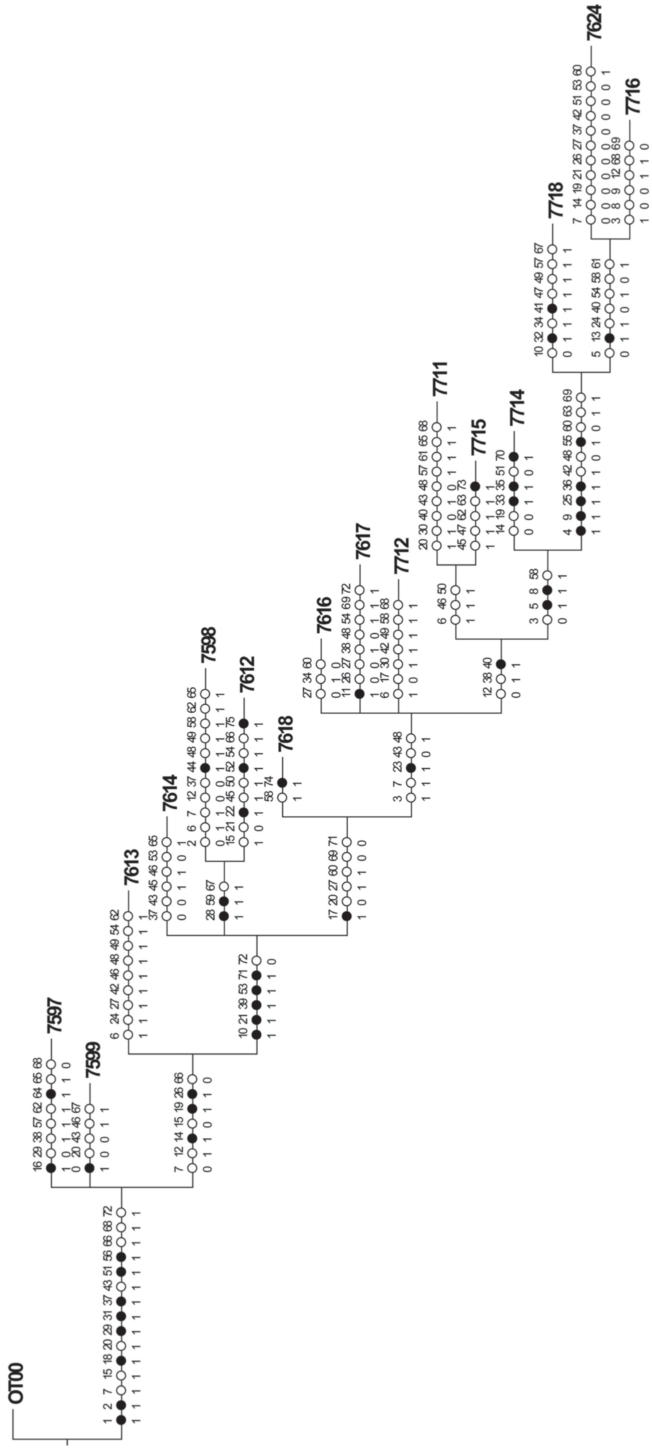


Fig. 7. Strict consensus cladogram (fast optimization) resulting from PAE by ottobasins. The white circles correspond to homoplasies, and the black circles denote species that occur exclusively in this area. The number above the circles represents species code; number below the circles means present (1) or absent (0).

Itabapoana river (7718) presented two exclusive taxa (*Paracloeodes* sp. 1 and *L. inanis*), that together with the ottobasins of Itapemirim (7716) and José Pedro (7624) rivers had the unique taxa *Americabaetis titthion* Lugo-Ortiz & McCafferty, 1996; *Baetodes* sp. 2; *Cloeodes itajara* Massariol & Salles, 2011; *Tupiara ibirapitanga* Salles, Lugo-Ortiz, Da-Silva & Francischetti, 2003; and *Askola froehlichi* Peters, 1969. The basins mentioned above, *i.e.* (7716+7624+7718), along with the Jucu river basin (7714) shared two exclusive species: *Americabaetis* sp. 1 and *Baetodes* sp. 1. Furthermore, in the Jucu river basin (7714) three exclusive taxa were found: *Paracloeodes* sp. 2, *Spiritiops* sp. 1, and *Thraulodes* sp. 1.

Network Analysis Method. After the analyses of 658 records, NAM recognised four UCs and six diads (Table IV, Figs. 10–20) after the removal of 31 intermediary species and eight isolated species.

UC1 is composed of the distribution area of eight species: *As. froehlichi*; *Am. titthion*; *Baetodes serratus* Needham & Murphy, 1924; *Baetodes* sp. 2; *Co. itajara*; *L. inanis*; *Paracloeodes* sp. 1; and *Tu. ibirapitanga*. The spatial expression of this unit matches the *Parque Nacional do Caparaó*, including the collection points PT 01, 02, 03, 14, and 15 (Fig. 17).

UC2 comprises the distribution areas of four species: *Americabaetis* sp. 1, *Spiritiops* sp. 1, *Thraulodes* sp. 1, and *Tricorythodes santarita* Traver, 1959. This unit is in the cen-

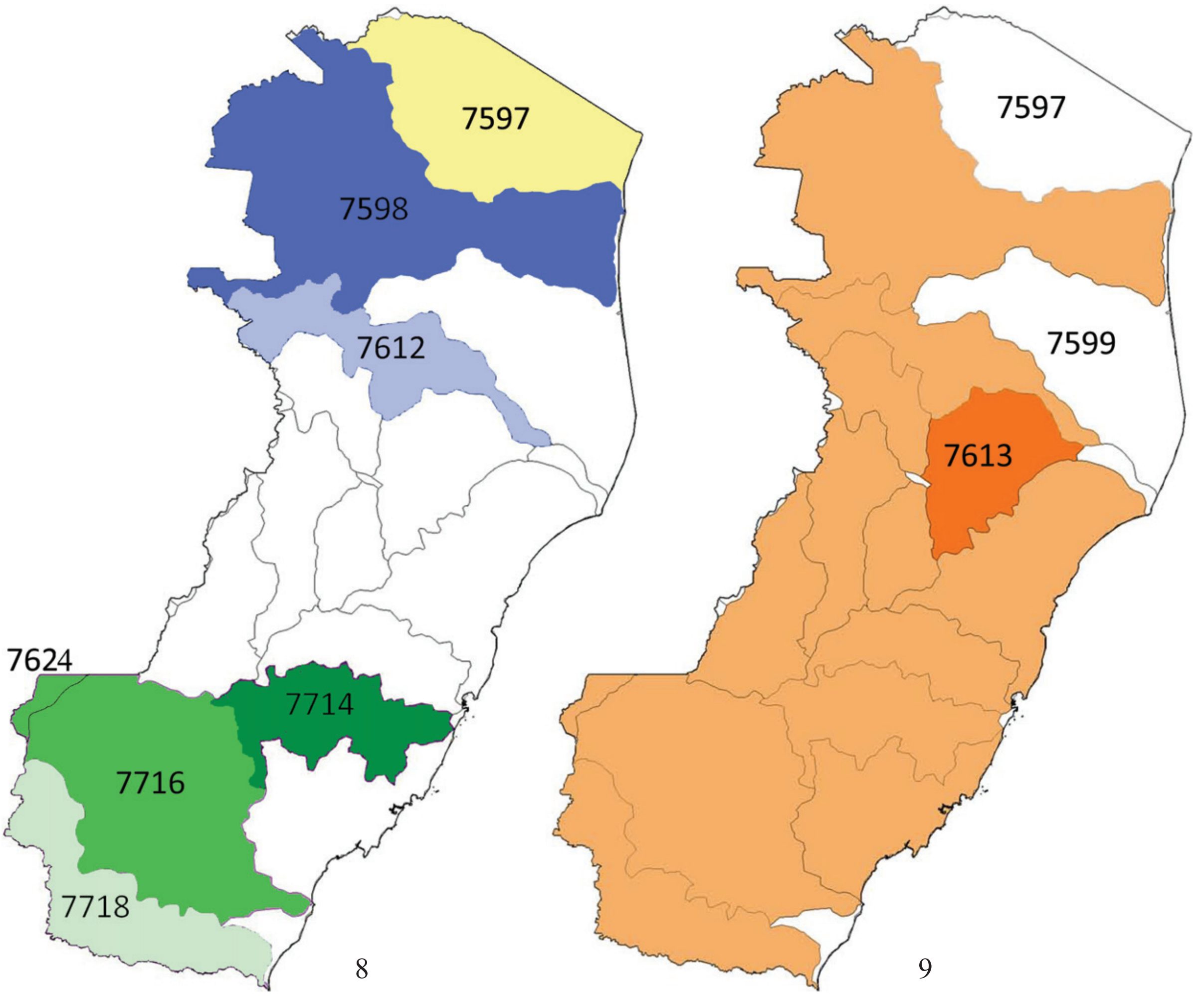
Table II. Matrix with the distribution data of 76 Ephemeroptera species at 48 collection points.

		1	2	3	4	5	6	7
	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	012345
PT 00	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	000000
PT 01	0010100000	0001000000	0001100000	0000000000	0000000000	0000010000	0001000000	000000
PT 02	0110100011	0000000110	0001010001	0010001010	0100000000	0001010000	0001000001	000000
PT 03	0010100000	0001000000	0001010001	0000001010	0000000000	0000010000	0101000000	000000
PT 04	0111000000	1000000011	1100001101	1100000110	0000000000	0101001000	0000000010	000000
PT 05	0110001100	1010100011	0100000101	1100000100	0010000010	0101001000	0000000010	000000
PT 06	0110001100	0000100011	0100001001	1100000100	0001001000	0100001100	0000010010	000000
PT 07	0110001000	0010100011	0000101100	0100000100	0001000010	0100101000	0010000010	000000
PT 08	0100000000	0000001010	1000000000	0100000010	0001000000	0100001000	0010101000	001000
PT 09	0110000100	0000010010	0000000001	0100000000	0000000000	0000001000	0000000010	001000
PT 10	0100001100	0000100010	0000001011	0100000000	0000100010	0101001000	0010010110	010000
PT 11	0110000010	1000000010	0101000100	0100000011	0000000000	0000001000	1000000000	000000
PT 12	0100000000	0010100011	0100001100	0100000100	0000000000	0100001000	0000000000	000000
PT 13	0111000100	1000000111	0100001101	0100000101	1000000010	1100001000	1000000000	000000
PT 14	0110000011	1001000110	0001000001	0100000011	0000000000	0000001000	1000000001	000000
PT 15	0100000010	0000000100	0000010001	0100001011	0000000000	0000101000	0101000001	000000
PT 16	0100000000	1010000010	0000001001	0100000101	0000000000	0001001000	1000000000	000010
PT 17	0100000000	0000100110	0100001101	0100000001	0001000000	0101001010	0000000000	000000
PT 18	0110000000	0010000011	0000000000	0000000001	0000000000	0000001000	0000000000	000000
PT 19	0100000100	0000100010	1000000001	0100000000	0000000000	0000001001	0000000010	010000
PT 20	0101000100	1010000110	0100101101	0100000111	0010000000	0001101000	0000000010	000000
PT 21	0101000000	1000000111	0001001101	0100000111	0000001110	1001001000	1011000000	000100
PT 22	0111000000	1010000111	0101000001	0100000101	0000000000	0001001000	1000000000	000000
PT 23	0101000100	1000100011	0101001101	0100000101	0000000001	0001001010	0000000000	000000
PT 24	0100000000	1010000011	0000000000	0100000101	0001000000	1101101001	0000000000	000000
PT 25	0100000000	0000010010	0000000001	0100000000	0000000000	0000000000	0000000000	000000
PT 26	0100000000	0000000010	1000000001	0100000000	0000011000	0100001000	0000010010	010000
PT 27	0110000000	0000100010	1010001011	0100000000	0001010000	0110001001	0000001110	010001
PT 28	0100000000	1000000011	0100000000	0100000001	0001000001	0001001010	0000000000	000000
PT 29	0110000000	0000000010	0000000001	0000000000	0000000000	0000001000	0000000000	001000
PT 30	0110000100	0000010010	0000000000	0100000000	0001000000	0100001100	0000010000	000000
PT 31	0110001000	0000100010	0100001100	0100000000	0000010000	0101001000	1000000000	000000
PT 32	0010000000	1000000000	0100000000	0000000001	0000000000	0000000000	0000000000	000000
PT 33	0110000000	0000100010	1000000100	0100000100	0011001001	0100001000	0000000010	000000
PT 34	0110000000	1010100011	0100001001	0100000001	0000000000	0100001000	0000000000	000000
PT 35	1100000000	0000000010	0000000000	0100000100	0000001000	0100001000	0000001100	001000
PT 36	0110000100	0000100000	0000000000	0100000010	0000000000	0101101000	1000000001	001000
PT 37	0110000000	0100000011	0100000000	0000000000	0000000000	0000001000	0000000000	000000
PT 38	0110000000	1000000110	0101001101	0100000011	0000000000	1101001000	1100000000	000000
PT 39	0100010100	0000100010	0100001101	0100100101	1010000001	0101001010	0000000000	000000
PT 40	0110000100	0000100111	0100001101	0100000100	0010000100	0100001100	0000000100	000000
PT 41	0110010100	0000000110	0001000001	0101000100	0000000000	0000000000	0000000000	000000
PT 42	0110000000	1000000010	0100001101	0100010001	1000000010	0001001010	0000000000	100000
PT 43	0100000000	0010000110	0000001000	0100000101	0000000010	0001001000	0000000000	000000
PT 44	0100000000	1000100011	0101001000	0000000000	0000000000	0000001000	1000000000	000000
PT 45	0100000000	1000000110	0101000000	0000100001	0000000000	0001001000	0000000000	000000
PT 46	0111000100	0000100011	0000000001	0100000100	0000000000	0100001000	0000000000	000000
PT 47	0100000100	0000010000	0000000000	0100000100	0000000000	0000000000	0000000000	001000
PT 48	0100000100	0000010000	0000000000	0000000100	0000000000	0000000000	0000000000	001000

tral, north coast, and northwest regions of Espírito Santo and encompasses the collect points PT 26, 27, 31, 42, and 46 (Fig. 18).

UC3 encompasses the distribution area of seven species: *Ad. lugoi*, *Hy. obliquus*, *Cm. juparana*, *O. amandae*, *Ti. spongicola*, *Ti. rondoniensis*, and *Ha. gulosus*. This unit is in the north coast and northwest regions and encompasses the collection points PT 10, 19, 24, 27, and 35 (Fig. 19). UCs 2 and 3 overlap at one point, PT 27 (Sooretama, São José river), which has exclusive taxa of the two units.

UC4 is composed of the distribution area of six species: *Baetodes santatereza* Salles & Polegatto, 2008; *Callibaetis* sp. 1; *Cm. francischettii*; *Tricorythopsis gibbus* (Allen, 1967); *Tricorythopsis araponga* Dias & Salles, 2005; and *W.*



Figs. 8–9. Spatial expression of ottobasins resulting from PAE. 8. Ottobasin in yellow (7597) has two exclusive species. Ottobasin in light blue (7612) has three exclusive taxa, and in conjunction with that in dark blue (7598) have two exclusive species. Ottobasin in light green (7718) has two exclusive taxa, jointly with those in green (7716, 7624) have five exclusive taxa. Ottobasin in dark green showed three exclusive species. The four ottobasins in green (light green, green, and dark green) have two exclusive species.

Table III. Matrix with the distribution data of 76 Ephemeroptera species in 16 ottobasins.

		1	2	3	4	5	6	7
	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	0123456789	012345
OT00	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	000000
7597	0110000100	0000011010	1000000000	0100000110	0001000000	0100001100	0010111000	001000
7598	0100001100	1000100011	1100001011	0100000001	0001100011	0101001011	0010010110	010000
7599	1110000100	0000010010	0000000001	0100000100	0000001000	0100001000	0000001110	001000
7612	0110000000	1010110011	1010001011	0100000101	0001010000	1111101001	0000001110	010001
7613	0110001000	0010100011	1000101101	0100000100	0011001011	0100101000	0010000010	001000
7614	0110000000	1010100011	1100001001	0100000001	0000011000	0100001000	0000010010	010000
7616	0111000100	1010100111	0101001001	0100100101	0000000010	0101001000	0000000000	000000
7617	0111000100	1110100111	0101000001	0100000111	0000000000	0101101000	1000000001	001000
7618	0110000000	1010100111	0100001101	0100000101	0001000000	0101001010	1000000000	000010
7624	0110100011	1001000110	0001110001	0100001011	0000000000	0000111000	1101000001	000000
7711	0111001100	1000100111	1101001101	1100000111	0001001000	1101001100	1100010010	000000
7712	0111001100	1010100011	0101001101	1100000101	0010000011	0101001010	1000000010	000000
7714	0110010110	1000000110	0101001101	0101010111	1000000010	0001001010	1000000000	100000
7715	0111001100	1000100111	0101001101	0100000111	1000011110	1101001000	1011000000	000100
7716	0111100100	1011100111	0101111101	0100001111	0010000000	0101111000	0101000010	000000
7718	0110110111	0000100111	0101011101	0110101111	1110000101	0101011110	0001000101	000000

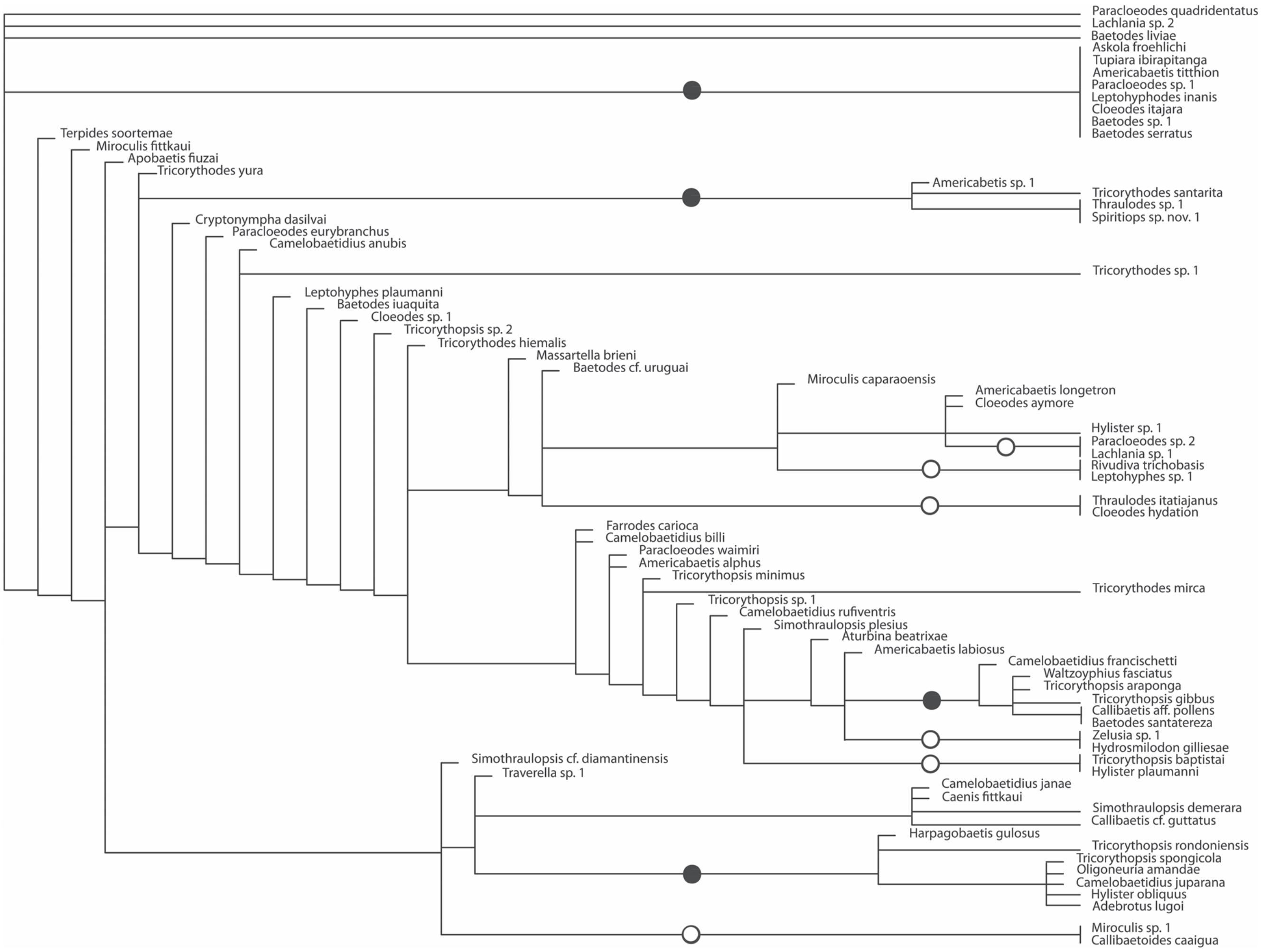


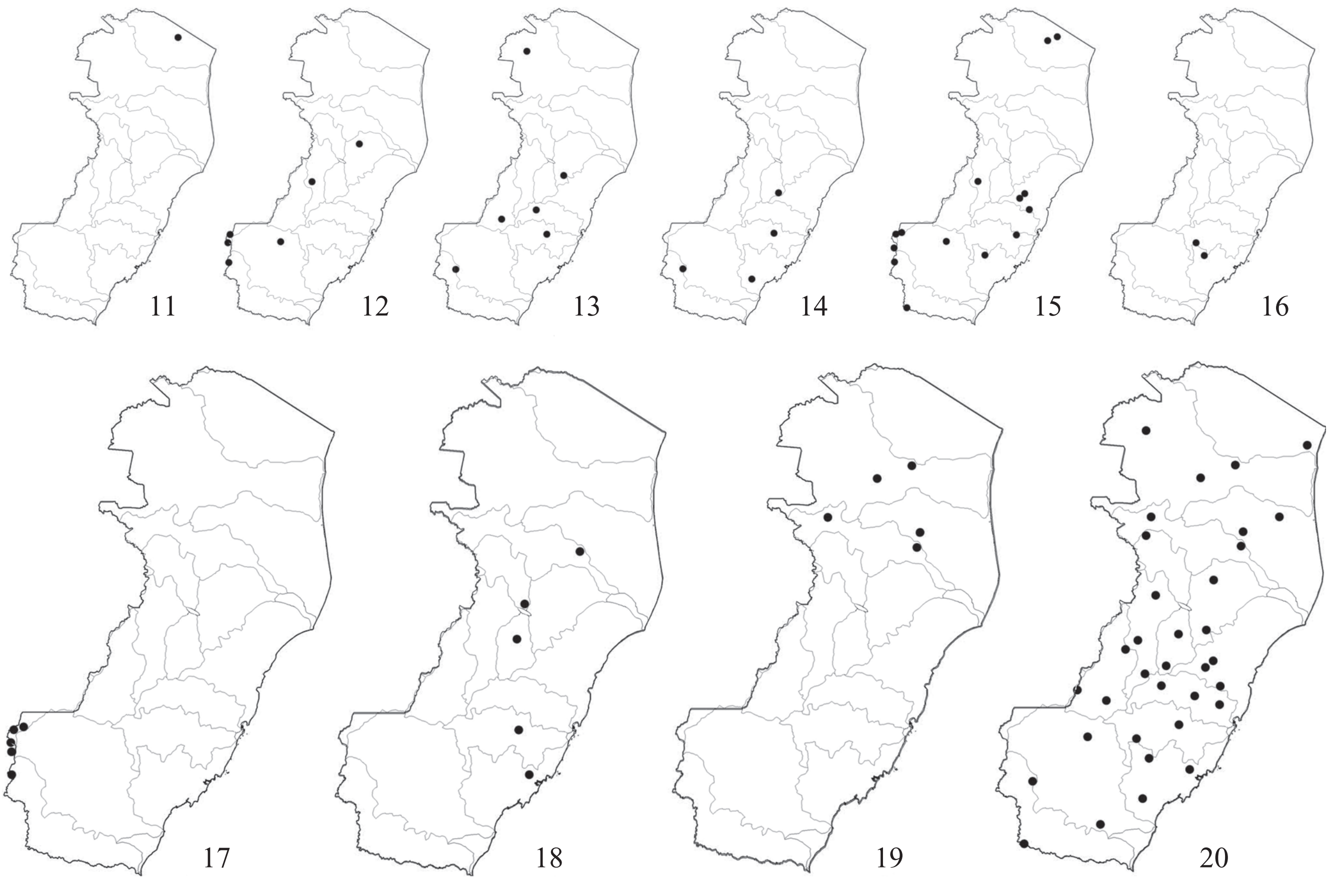
Fig. 10. Cleavogram showing the sequence of the segregation of groups through the removal of intermediary species. The units of co-occurrence are represented by black dots and diads by white dots.

Table IV. Diads and units of co-occurrence (UCs) found using NAM, followed by the corresponding species.

Diads and UCs	Species
Diad 1	*Callibaetoides caaigua*, *Miroculis* sp. 1
Diad 2	*Cloeodes hydation*, *Thraulodes itatiajanus*
Diad 3	*Hylister plaumanni*, *Tricorythopsis baptistai*
Diad 4	*Leptohyphes* sp. 1, *Rivudiva trichobasis*
Diad 5	*Hydrosmilodon gilliesae*, *Zelusia* sp. 1
Diad 6	*Lachlania* sp. 1, *Paracloeodes* sp. 2
UC 1	*Americabaetis titthion*, *Askola froehlichi*, *Baetodes serratus*, *Baetodes* sp. 1, *Cloeodes itajara*, *Leptohyphodes inanis*, *Paracloeodes* sp. 1, *Tupiara ibirapitanga*
UC 2	*Americabaetis* sp. 1, *Spiritiops* sp. 1, *Thraulodes* sp. 1, *Tricorythodes santarita*
UC 3	*Adebrotus lugoi*, *Camelobaetidius juparana*, *Harpagobaetis gulosus*, *Hylister obliquus*, *Oligoneuria amandae*, *Tricorythopsis spongicola*, *Tricorythopsis rondoniensis*
UC 4	*Baetodes santatereza*, *Callibaetis* sp. 1, *Camelobaetidus francischetti*, *Tricorythopsis araponga*, *Tricorythopsis gibbus*, *Waltzoyphius fasciatus*

fasciatus. This unit has a very broad spatial expression, with points from north to south (Fig. 20).

Diads 2, 3, and 5 showed a broad spatial expression in the state (Figs. 12, 13, and 15) and are composed of the distribution area of *Cloeodes hydation* McCafferty & Lugo-Ortiz, 1995 – *Thraulodes itatiajanus* Traver & Edmunds, 1967; *Hylister plaumanni* Dominguez & Flowers, 1989 – *Tricorythopsis baptistai* Dias & Salles, 2005; and *Hydrosmilodon gilliesae* Thomas & Péru, 2004 – *Zelusia* sp. 1, respectively. The remaining diads have a more restricted spatial expression: diad 1 with spatial expression at only one point in the northern state with exclusive taxa *Cl. caaigua*, and *Miroculis* sp. 1 (Fig. 11); diad 4 (Fig. 14) and 6 (Fig. 16) both restricted to the south with exclusive taxa *Rivudiva trichobasis* Lugo-Ortiz & McCafferty, 1998 – *Leptohyphes* sp. 1, and *Lachlania* sp. 1 – *Paracloeodes* sp. 2, respectively.

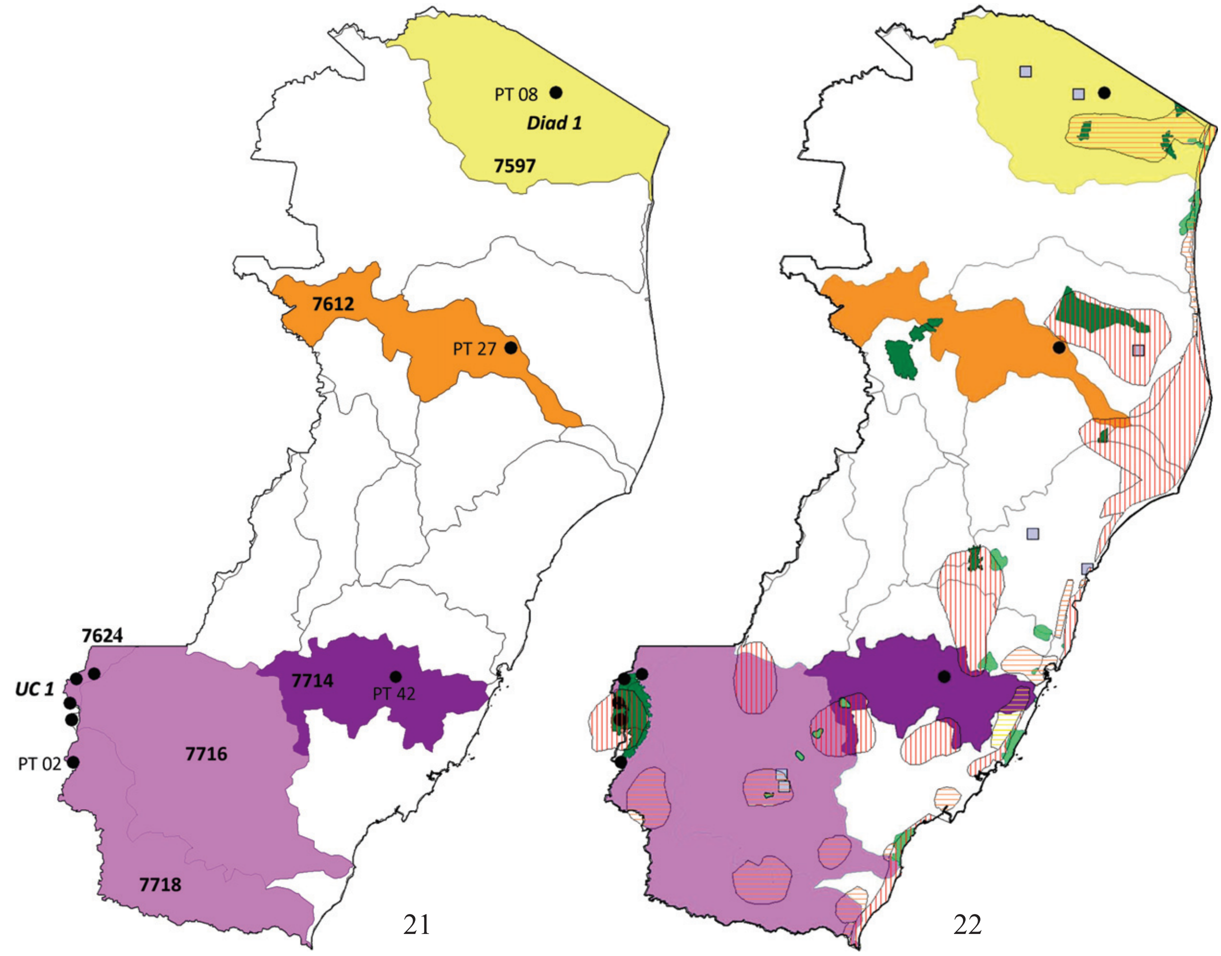


Figs. 11–20. Spatial expression of UCs and diads resulting from NAM. 11. Diad 1. 12. Diad 2. 13. Diad 3. 14. Diad 4. 15. Diad 5. 16. Diad 6. 17. UC 1. 18. UC 2. 19. UC 3. 20. UC 4.

DISCUSSION

The results of the three analyses were largely congruent, particularly the results of the two approaches using PAE. Considering the total congruence in at least two of the three analyses, the following four relevant areas for conservation of the mayfly community in Espírito Santo were inferred (Fig. 21). Area 1: In the extreme north, this area corresponds to PT 08, ottobasin 7597 (interbasin of the Itaúnas river), and diad 10 and comprises the distribution of two exclusive species: *Cl. caaigua* and *Miroculis* sp. 1. Area 2: On the north coast and in the northwest region, this area corresponds to PT 27 and ottobasin 7612 (basin of the São José river) and encompasses the distribution of three exclusive species: *Cm. juparana*, *Ti. spongicola*, and *O. amandae*. Although there was no total congruence between the results of PAE and NAM for this area, PT 27 in the Network Analysis was a point of overlap for two UCs (UC2 and UC3). Area 3: In the central region, this area corresponds to PT 42 and ottobasin 7714 (basin of Jucu river) and is composed of the distribution of three exclusive species: *Paracloeodes* sp. 2, *Spiritiops* sp. 1, and *Thraulodes* sp.1. Area 4: In the south along the border with Minas Gerais, this area corresponds to ottobasins 7718, 7624, and 7716 (the basins of the Itabapoana, José Pedro, and Itapemirim rivers, respectively), containing PT 02 and UC1. This area includes eight exclusive species: *As. froehlichi*, *Am. titthion*, *B. serratus*, *Baetodes* sp. 2, *Co. itajara*, *L. inanis*, *Paracloeodes* sp. 1, and *Tu. ibirapitanga*.

The inferred areas for conservation 1 and 4 have the largest number of previously protected areas, with six conservation units each (Fig. 22). Area 1 presents units with a small size (not exceeding 3,000 ha), whereas area 4 contains *Parque Nacional do Caparaó*, the second largest unit of conservation in Espírito Santo and five other small units. Area 1 is located in the north of the state, a region with few forest fragments due to the severe deforestation occurred in the 19th century by wood exploration and actually by vast eucalyptus plantations (IPEMA 2005; Paula 2006). It is noteworthy that the spatial expression of UC1 corresponds to the region of *Parque Nacional do Caparaó*. Area 2 encompasses only a small part of *Parque Nacional dos Pontões Capixabas*, and area 3 contains only the *Parque Estadual de Pedra Azul* (Fig. 22); both areas present three exclusive taxa, but the number as well as the size of conservation units area are minimal (not exceeding 1,300 ha). The point PT 27 located in area 2 (corresponding to ottobasin 7612), showed the highest number of exclusive species, besides in NAM analyses PT 27 was a point of contact between UCs 2 and 3, so this area has



Figs. 21–22. Spatial expression of the congruence of the results of three analyses showing four relevant areas for conservation of the mayfly community. 21. Area 1 – yellow; area 2 – orange, area 3 – purple, area 4 – light purple. PT 02, 08, 27, and 42 resulting from PAE by collection point; 7597, 7612, 7714, 7716, 7718, and 7624 resulting from PAE by ottobasin, and diad 1 and UC 1 by NAM. 22. Relevant areas for conservation of the mayfly community in Espírito Santo plotted on existing conservation units and priority areas for conservation by MMA. Federal conservation units are in dark green, state in light green, and private reserve in light blue. Red vertical stripes, APCs with extremely high priority; orange horizontal stripes with very high priority; yellow horizontal stripes with high priority.

a high complexity. Despite the ottobasin 7612 being an area that suffer intense anthropogenic pressure (ANA 2001) and be recognized (through PAE and NAM) as one of the relevant areas for conservation, it is unprotected, with only a small part set as a conservation unit in western state. When we compared the inferred areas for conservation (Fig. 21) with APCs of MMA (Fig. 4), we observed that most APCs are in areas 3 and 4 in the southern part of state (Fig. 22). Although area 3 has four APCs all are located marginally. The area 4 is extensive (ottobasins 7624, 7716 and 7718), however the points of exclusive taxa are concentrated in a small area, so the methods showed that this area can be a natural unit. The APCs in area 4 seem to be sufficient and necessary to preserve the biodiversity of the studied group, because they are well distributed spatially, and covers the points of exclusive taxa resulting by PAE and NAM analyses. The contact area between areas 3 and 4 was previously indicated as extremely high priority for conservation. In this work the data suggested that this area is complex, because it is a transition between two areas, so our data corroborate the status given by the MMA assessment.

Unlike the southern portion of the state, northern Espírito Santo is poorly represented by APCs. In this work two areas were pointed out in the north of the state, however only one APC was indicated by MMA, and it is located on area 1. Despite the area 2 being indicated in this study as a relevant area for conservation of the mayfly community and with a high complexity, the MMA did not pointed out any area within this region as APC. This fact occurs because of two historical and probably dependent reasons: little effort has been done in the north of the state in order to uncover its biodiversity; and, except for *Reserva Biológica de Sooretama* and *Reserva da Vale*, only small fragments of forest persist in this area due to agricultural activities. So, this situation makes the region to receive less interest from researches (Moreira *et al.* 2008).

The north coast of the state is widely suggested as having high priority for conservation according to MMA, however the western portion is unprotected by both the lack of conservation units and APCs. Furthermore, the point of contact between UCs 2 and 3 (PT 27) is unprotected by conservation units, and it is not identified as a priority for conservation by MMA. All areas resulting from the analyses present at least one conservation unit or one portion of APC with the exception of point PT 27 in the north of Espírito Santo. Therefore, it is recommended that the existing APCs be expanded to integrate the relevant areas for conservation of the mayfly community inferred in this work, or the creation of new APCs particularly on the north of Espírito Santo.

ACKNOWLEDGMENTS

We are grateful to *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, grants 402939/2012–3, 245924/2012–4) and *Fundação de Apoio à Ciência e Tecnologia do Espírito Santo* (FAPES, grants 54689627/2011, 511187434/2010) for financial support; IBAMA (I*nstituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis*) for collecting permits; Ezinete Moreira do Rozário for her invaluable support with the fieldwork; to *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for scholarship provided to the first author and to CNPq for research scholarship provided to the third author (grant 306670/2012-7). To Carlos Molineri for his help with biogeographic methods, and to Luisa Maria Soares Porto and Mateus Pepinelli for their contributions to dissertations that originated this work.

REFERENCES

ANA – Agência Nacional de Águas. 2001. Bacias Hidrográficas do Atlântico Sul – trecho leste. Sinopse de informações do Rio de Janeiro, Espírito Santo, Bahia e Sergipe. Série: Sistema Nacional de Informações sobre Recursos Hídricos – CD n. 4. Available at: http://hidroweb.ana.gov.br/doc/BHASLeste/index.htm (accessed 10 February 2014).

ANA – Agência Nacional de Águas. 2006. Topologia Hídrica: Método de Construção e Modelagem da Base Hidrográfica para Suporte à Gestão de Recursos Hídricos – Versão 1.11. Brasília, ANA – Agência Nacional de Águas, SGI – Superintendência de Gestão da Informação, 29 p.

Baptista, D.F., Buss, D.F., Dorvillé, L.F.M. & Nessimian, J.L. 2001. Diversity and habitat preference of aquatic insects along the longitudinal gradient of the Macaé River Basin, Rio de Janeiro, Brazil. **Revista Brasileira de Biologia 61**: 249–258.

Baptista, D.F., Buss, D.F., Egler, M., Giovanelli, A., Silveira, M.P. & Nessimian, J.L. 2007. A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. **Hydrobiologia 575**: 83–94.

Beketov, M.A. 2004. Different sensitivity of mayflies (Insecta, Ephemeroptera) to ammonia, nitrite and nitrate: linkage between experimental and observational data. **Hydrobiologia 528**: 209–216.

BRASIL. 1988. **Constituição da República Federativa do Brasil**. Texto constitucional promulgado em 5 de outubro de 1988, com as alterações adotadas pelas Emendas Constitucionais nos 1/1992 a 68/2011, pelo Decreto Legislativo no186/2008 e pelas Emendas Constitucionais de Revisão nos 1 a 6/1994, Brasília.

Britain, J.E. & Sartori, M. 2003. Ephemeroptera (Mayflies), p. 373–380. In: Resh, V.H. & Cardé, R.T. (Eds.). **Encyclopedia of Insects**. California, Academic Press, an imprint of Elsevier Science, 1295 p.

Buss, D.F. & Salles, F.F. 2007. Using Baetidae species as biological indicators of environmental degradation in a Brazilian river basin. **Environmental Monitoring and Assessment 130**: 365–372.

Carvalho, C.J.B. 2004. Ferramentas atuais da Biogeografia histórica para utilização em conservação, p. 92–103. In: Milano, M.S., Takahashi, L.Y. & Nunes, M.L. (Eds.). **Unidades de Conservação: atualidades e tendências**. Curitiba, Fundação o Boticário de Proteção da Natureza, 224 p.

Cavieres, L.A., Arroyo, M.T.K., Posadas, P., Marticorena, C., Matthei, O., Rodríguez, R., Squeo, F.A. & Arancio, G. 2002. Identification on priority areas for conservation in an arid zone: application of parsimony analysis of endemicity in the vascular flora of the Antofagasta region, northern Chile. **Biodiversity and Conservation 11**: 1301–1311.

Chen, Y. & Bi, J. 2007. Biogeography and hotspots of amphibian species of China: Implications to reserve selection and conservation. **Research Articles 92**: 480–489.

Clark, J.R. 1999. The ecosystem approach from a practical point of view. **Conservation Biology 13**: 679–681.

Clark, J.A. & May, R.M. 2002. Taxonomic bias in conservation research. **Science 297**: 191–192.

Crisci-Bispo, V.L., Bispo, P.C. & Froehlich, C.G. 2007. Ephemeroptera, Plecoptera and Trichoptera assemblages in litter in a mountain stream of the Atlantic Rainforest from Southeastern Brazil. **Revista Brasileira de Zoologia 24**: 545–551.

Domínguez, E. & Valdez, J.M.B. 1992. Altitudinal replacement of Ephemeroptera in a subtropical river. **Hydrobiologia 246**: 83–88.

Domínguez, E., Molineri, C., Pescador, MI., Hubbard, M.D. & Nieto, C. 2006. Ephemeroptera of South America. In: Adis, J.R., Rueda-Delgado, G & Wantzen, K.W. (eds.) **Aquatic Biodiversity of Latin America**. Vol. 2, Sofia, Pensoft Publishers, 646 p.

Dos Santos, D.A., Fernández, H.R., Cuezzo, M.G. & Domínguez, E. 2008. Sympatry inference and network analysis in Biogeography. **Systematic Biology 57**: 432–448.

Dos Santos, D.A., Cuezzo, M.G., Reynaga, M.C. & Domínguez, E. 2012. Towards a dynamic analysis of weighted networks in Biogeography. **Systematic Biology 61**: 240–252.

Edmunds, G.F., Jr., Jensen, S.L. & Boner, L. 1976. The biological role of mayflies, p. 3–50. In: Edmunds, G.F., Jr., Jensen, S.L. & Boner, L. (Eds.). **The mayflies of North and Central America**. Minneapolis, University of Minnesota Press, x+330 p.

Fischer, J. & Lindenmayer, D.B. 2007. Landscape modification and habitat fragmentation: a synthesis. **Global Ecology and Biogeography 16**: 265–280.

Gallardo-Mayenco, A. 2003. Distribuición espacial de los efemerópteros (Insecta: Ephemeroptera) en dos cuencas mediterráneas a diferentes altitudes. **Zoological Baetica 13/14**: 93–110.

Goloboff, P., Farris, S. & Nixon, K. 2004. T.N.T. – Tree analysis using new technology. Available at: http://www.cladistics.com (accessed 05 December 2012).

Hickey, C.W. & Clements, W.H. 1998. Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. **Environmental Toxicology and Chemistry 17**: 2338–2346.

Hijmans, R.J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I. & Rojas, E. 2005. DIVA-GIS: A geographic information system for the analysis of species distribution data, version 5.2. Available at: http://www.diva-gis.org (accessed 05 December 2012).

Huang, X.-L., Qiao, G.-X. & Lei, F.-M. 2010. Use of parsimony analysis to identify areas of endemism of Chinese birds: Implications for conservation and biogeography. **International Journal of Molecular Sciences 11**: 2097–2108.

IJSN – Instituto Jones dos Santos Neves. 2009. Ottobacias Hidrográficas do Espírito Santo – Nível 04. Espírito Santo. Escala 1: 2.000.000.

IPEMA – Instituto de Pesquisas da Mata Atlântica. 2005. **Conservação da Mata Atlântica no Estado do Espírito Santo: Cobertura florestal e Unidades de Conservação**. Secretaria de Meio Ambiente e Recursos Hídricos, Espírito Santo, 152 p.

Lenat, D.R. & Barbour, M.T. 1994. Using benthic macroinvertebrate community structure for rapid, cost-effective, water quality monitoring: Rapid bioassessment, p. 187–215. In: Loeb, S.L. & Spacie, A. (Eds.). **Biological Monitoring of Aquatic Systems.** 1st ed. Washington DC, Lewis Publishers, 400 p.

Löwenberg-Neto, P. 2011. Conservação da biodiversidade e biogeografia histórica, p. 162–172. In: Carvalho, C.J.B. & Almeida, E.A.B. (Eds.). **Biogeografia da América do Sul – Padrões e Processos**. São Paulo, Editora Roca, 306 p.

Medeiros, R., Young, C.E.F., Pavese, H.B. & Araújo, F.F.S. 2011. Unidades de conservação e sua importância para a conservação da biodiversidade, p. 8–12. In: Medeiros, R., Young, C.E.F., Pavese, H.B & Araújo, F.F.S. (Eds.). **Contribuição das Unidades de Conservação para a Economia Nacional: Sumário Executivo**. Brasília, UNEP-WCMC, 44 p.

MMA – Ministério do Meio Ambiente. 2000. **Avaliação e ações prioritárias para a conservação da biodiversidade da Mata Atlântica e Campos Sulinos**. Conservation International do Brasil/Fundação SOS Mata Atlântica/Fundação Biodiversitas/Instituto de Pesquisas Ecológicas/ Secretaria do Meio Ambiente do Estado de São Paulo SEMAD/Instituto Estadual de Florestas-MG, Brasília, 45 p.

Moreira, D.O., Coutinho, B.R. & Mendes, S.L. 2008. O status do conhecimento sobre a fauna de mamíferos do Espírito Santo baseado em registros de museus e literatura científica. **Biota Neotropica 8**: 163–173.

Morrone, J.J. 2014. Parsimony analysis of endemicity (PAE) revisited. **Journal of Biogeography 41**: 842-854.

Moulton, T.P. & Souza, M.L. 2006. Conservação com base em bacias hidrográficas, p. 157–182. In: Rocha, C.F.D., Bergallo, H.G., Sluys, M.V. & Alves, M.A.S. (Eds.). **Biologia da Conservação: Essências**. São Carlos, RiMa Editora, 582 p.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. **Nature 403**: 853–858.

Nelson, G. & Platnick, N.I. 1981. **Systematics and biogeography: cladistics and vicariance**. Columbia Press University, xi+567 p.

Nixon, K.C. 1999. Winclada versão 1.0. Available at: http://www.cladistics.com/about_winc.htm (accessed 05 December 2012).

Passamani, M. 2007. Situação atual da fauna ameaçada de extinção no Estado do Espírito Santo, p. 15–20. In: Passamani, M. & Mendes, S.L. (Orgs.). **Espécies da Fauna Ameaçadas de Extinção no Estado do Espírito Santo**. Vitória, IPEMA – Instituto de Pesquisas da Mata Atlântica, 140 p.

Paula, A. 2006. Espírito Santo, p. 114–128. In: Campanili, M. & Prochnow, M. (Eds.). **Mata Atlântica – Uma rede pela floresta**. Brasília, Rede ONGs pela Mata Atlântica, RMA, 334 p.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/(accessed 05 December 2012).

Resh, V.H. 1994. Variability, accuracy, and taxonomic costs of rapid assessment approaches in benthic macroinvertebrate biomonitoring. **Bolettino di Zoologia 61**: 375–383.

Salles, F.F. 2006. A ordem Ephemeroptera no Brasil (Insecta): Taxonomia e diversidade. Ph. D. Dissertation, Universidade Federal de Viçosa, 313 p.

Salles, F.F., Nascimento, J.M.C., Massariol, F.C., Angeli, K.B., Silva, P.B., Rúdio, J.A. & Boldrini, R. 2010. Primeiro levantamento da fauna de Ephemeroptera (Insecta) do Espírito Santo, Sudeste do Brasil. **Biota Neotropica 10**: 293–307.

Soulé, M.E. & Simberloff, D. 1986. What do genetics and ecology tell us about the design of nature reserver? **Biological Conservation 35**: 19–40.

Tierney, L. 2010. tkrplot: Tk R Plot. R package version 0.0-19. Available at: http://CRAN.R-project.org/package = tkrplot (accessed 05 December 2012).

Waltz, R.D. & Burian, S.K. 2008. Ephemeroptera, p. 181–236. In: Merrit, R.W., Cummins, K.W. & Berg, M.B. (Eds.). **An introduction to the aquatic insects of North America.** 4th ed. Kendall/Hunt Publishing Company, 1214 p.

Appendix 1. Species list with respective codes used for PAE and the collection points that taxa occurs. Species that occur exclusively in Espírito Santo are followed by an asterisk (*).

Species code	Taxa	Collection points (PT)
0	*Adebrotus lugoi** Salles, 2010	35
1	*Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996	02, 04-31, 33-48
2	*Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996	01-07, 09, 11, 13, 14, 18, 22, 27, 29, 30-34, 36-38, 40-42, 46
3	*Americabaetis longetron* Lugo-Ortiz & McCafferty, 1996	04, 13, 20-23, 46
4	*Americabaetis titthion* Lugo-Ortiz & McCafferty, 1996	01-03
5	*Americabaetis* sp. 1	39, 41
6	*Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002	05-07, 10, 31
7	*Aturbina beatrixae* Gillies, 2001	05, 06, 09, 10, 13, 19, 20, 23, 30, 36, 39-41, 46-48
8	*Baetodes* sp. 1	02, 11, 14, 15
9	*Baetodes* sp. 2	02, 14
10	*Baetodes iuaquita** Salles & Nessimian, 2011	04, 05, 11, 13, 14, 16, 20-24, 28, 32, 34, 38, 42, 44, 45
11	*Baetodes liviae* Polegatto & Salles, 2008	37
12	*Baetodes santatereza** Salles & Polegatto, 2008	05, 07, 12, 16, 18, 20, 22, 24, 34, 43
13	*Baetodes serratus* Needham & Murphy, 1924	01, 03, 14
14	*Callibaetis* sp. 1	05-07, 10, 12, 17, 19, 23, 27, 31, 33, 34, 36, 39, 40, 44, 46
15	*Callibaetis* sp. 2	09, 25, 30, 47, 48
16	*Callibaetoides caaigua* Cruz, Salles & Hamada, 2013	08
17	*Camelobaetidius anubis* (Traver & Edmunds, 1968)	02, 13-15, 17, 20-22, 38, 40, 41, 43, 45
18	*Camelobaetidius billi* Thomas & Dominique, 2000	02, 04-14, 16-31, 33-35, 37-46
19	*Camelobaetidius francischetti* Salles, Andrade & Da-Silva, 2005	04-07, 12, 13, 18, 21-24, 28, 34, 37, 40, 44, 46
20	*Camelobaetidius janae* Dominique & Thomas, 2000	04, 08, 19, 26, 27, 33
21	*Camelobaetidius rufiventris* Boldrini & Salles, 2009	04-06, 11-13, 17, 20, 22, 23, 28, 31, 32, 34, 37-40, 42, 44, 45
22	*Camelobaetidius juparana* Boldrini & Salles, 2012	27
23	*Cloeodes aymore** Massariol & Salles, 2011	01-03, 11, 14, 21-23, 38, 41, 44, 45
24	*Cloeodes hydation* McCafferty & Lugo-Ortiz, 1995	01, 07, 20

Continues

Appendix 1. Continued.

Species code	Taxa	Collection points (PT)
25	*Cloeodes itajara** Massariol & Salles, 2011	02, 03, 15
26	*Cloeodes* sp.1	04, 06, 07, 10, 12, 13, 16, 17, 20, 21, 23, 27, 31, 34, 38-40, 42-44
27	*Cryptonympha dasilvai* Lugo-Ortiz & McCafferty, 1998	04, 05, 07, 11-13, 17, 20, 21, 23, 31, 33, 38-40, 42
28	*Harpagobaetis gulosus* Mol, 1986	10, 27
29	*Paracloeodes eurybranchus* Lugo-Ortiz & McCafferty, 1996	02-06, 09, 10, 13-17, 19-23, 25-27, 29, 34, 38-42, 46
30	*Paracloeodes quadridentatus* Lima & Salles, 2010	04-06
31	*Paracloeodes wamiri* Nieto & Salles, 2006	04-17, 19-28, 30, 31, 33-36, 38-43, 46, 47
32	*Paracloeodes* sp. 1	02
33	*Paracloeodes* sp. 2	41
34	*Rivudiva trichobasis* Lugo-Ortiz & McCafferty, 1998	39, 45
35	*Spiritiops* sp. 1	42
36	*Tupiara ibirapitanga* Salles, Lugo-Ortiz, Da-Silva & Francischetti, 2003	02, 03, 15
37	*Waltzoyphius fasciatus* Lugo-Ortiz & McCafferty, 1995	04-07, 12, 13, 16, 20-24, 33, 35, 39-41, 43, 46-48
38	*Zelusia* sp. 1	02-04, 08, 11, 14, 15, 20, 21, 36, 38
39	*Leptohyphes plaumanni* Allen, 1967	11, 13-18, 20-24, 28, 32, 34, 38, 39, 42, 43, 45
40	*Leptohyphes* sp. 1	13, 39, 42
41	*Leptohyphodes inanis* (Pictet, 1843)	02
42	*Tricorythodes hiemalis* Molineri, 2001	05, 20, 33, 39, 40
43	*Tricorythodes mirca* Molineri, 2002	06-08, 17, 24, 27, 28, 30, 33
44	*Tricorythopsis rondoniensis* Dias, Cruz & Ferreira, 2009	10
45	*Tricorythodes santarita* Traver, 1959	26, 27, 31
46	*Tricorythodes yura* Molineri, 2002	06, 21, 26, 33, 35
47	*Tricorythodes* sp. 1	21, 40
48	*Tricorythopsis araponga* Dias & Salles, 2005	05, 07, 10, 13, 21, 42, 43
49	*Tricorythopsis baptistai* Dias & Salles, 2005	23, 28, 33, 39
50	*Tricorythopsis gibbus* (Allen, 1967)	13, 21, 24, 38
51	*Tricorythopsis minimus* (Allen, 1973)	04-08, 10, 12, 13, 17, 24, 26, 27, 30, 31, 33-36, 38-40, 46
52	*Tricorythopsis spongicola* Limas, Salles & Pinheiro, 2011	27
53	*Tricorythopsis* sp. 1	02, 04, 05, 10, 16, 17, 20-24, 28, 31, 36, 38, 39, 42, 43, 45
54	*Tricorythopsis* sp. 2	07, 15, 20, 24, 36
55	*Askola froehlichi* Peters, 1969	01-03
56	*Farrodes carioca* Dominguez, Molineri & Peters, 1996	04-24, 26-31, 33-40, 42-46
57	*Hydrosmilodon gilliesae* Thomas & Péru, 2004	06, 30, 40
58	*Hylister plaumanni* Dominguez & Flowers, 1989	17, 23, 28, 39, 42
59	*Hylister obliquus** Nascimento & Salles, 2013	19, 24, 27
60	*Hylister* sp. 1	11, 13, 14, 16, 21, 22, 31, 36, 38, 44
61	*Massartella brieni* (Lestage, 1924)	03, 15, 38
62	*Miroculis fittkaui* Savage & Peters, 1983	07, 08, 10, 21
63	*Miroculis caparaoensis** Salles & Lima, 2011	01-03, 15, 21
64	*Miroculis* sp. 1	08
65	*Simothraulopsis* (*Simothraulopsis*) *demerara* (Traver, 1947)	06, 10, 26, 30
66	*Simothraulopsis* sp. 1	08, 27, 35
67	*Simothraulopsis* (*Maculognathus*) *plesius* Kluge, 2008	10, 27, 35, 40
68	*Terpides sooretamae* Boldrini & Salles, 2009	04-07, 09, 10, 19, 20, 26, 27, 33
69	*Thraulodes itatiajanus* Traver & Edmunds, 1967	02, 14, 15, 36
70	*Thraulodes* sp. 1	42
71	*Traverella insolita** Nascimento & Salles, 2013	10, 19, 26, 27
72	*Caenis fittkaui* Malzacher, 1986	08, 09, 29, 35, 36, 47, 48
73	*Lachlania* sp. 1	21
74	*Lachlania* sp. 2	16
75	*Oligoneuria amandae** Salles, Soares, Massariol & Faria, 2013	27F

Received 31 March 2014; accepted 22 October 2014
Associate Editor: Gustavo Graciolli