



INTRODUCTION

Border Disease Virus (BDV), a pestivirus from the Flaviviridae family, is an important pathogen of sheep and goats responsible for significant losses in farms around the world. In spite of the relevance of this pathogen there are only a few epidemiological studies on BDV infection and, as a consequence, the economic impact on small ruminant productions is probably underestimated.

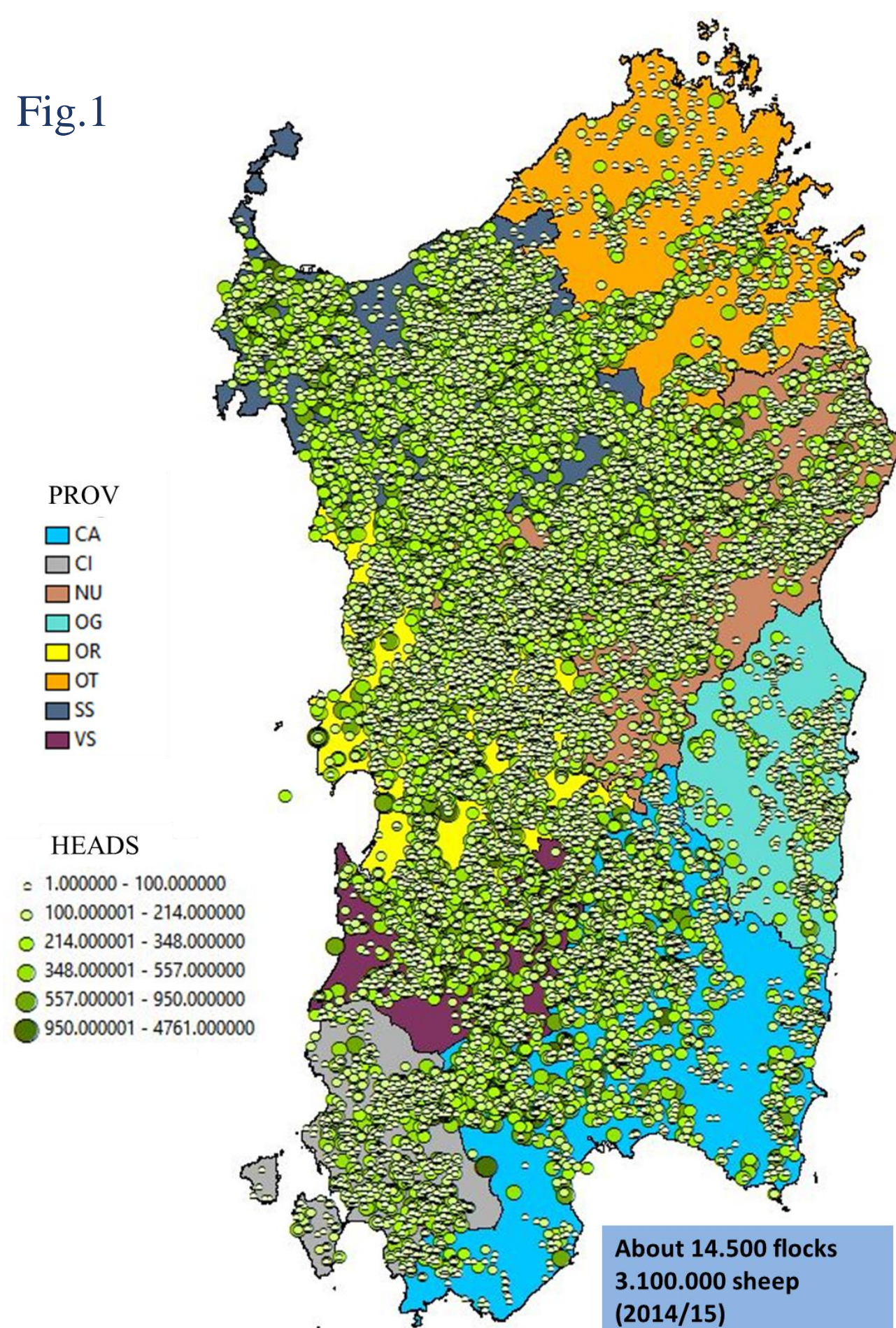
OBJECTIVES

The aims of this study are i) to determine the distribution of BDV in small ruminant farms in Sardinia ii) genetically characterize circulating strains iii) analyze the relation between seroprevalence and Somatic Cells Count (SCC).

MATERIAL & METHODS

ELISA was performed using "BVDV/MD/BDV p80 Protein Antibody Test Kit" (IDEXX) on serum from bulk tank milk (BTM) samples collected from 1196 Sardinian sheep flocks and 65 goat herds in 2014 and 2015. The number of sampled farms corresponded to 8,2% of all registered ones in Sardinia and sampling was performed according to their distribution in the region (Fig.1). RNA was isolated using Qiamp Viral RNA mini kit from the cellular fraction of each ELISA positive BTM sample and amplified by rt-PCR using complementary primers to a highly conserved region in the untranslated regions (UTRs) of the viral genome. The amplicons were sequenced for phylogenetic analysis. Geographic distribution of collected specimen, seroprevalence and virological positive samples was performed by GIS (ESRI ARCGIS 10.3)

Fig.1



RESULTS

Province	Total N° of flocks	Total N° of ewes	N° of tested flocks	N° of ewes	N° ELISA-antibody positive flocks	% ELISA-antibody positive flocks	N° PCR positive flocks
SS	3.228	843.301	232	71.935	12	5.17	1
OT	893	151.784	45	14.352	3	6.6	1
NU	3.348	725.170	342	85.673	17	4.97	0
OG	549	66.087	70	15.104	11	15.7	0
OR	2.715	518.955	179	56.741	19	10.6	1
VS	958	224.374	70	23.090	6	8.57	2
CI	898	147.007	71	12.688	1	1.4	0
CA	2.008	428.761	187	51.354	60	32.08	5
	14.597	3.105.439	1196	330.937	129		10

Tab.1 Number of flocks tested in the eight Sardinian provinces, number and percentage of antibody and PCR-positive flocks

Seropositivity shows a high degree of variations between provinces (see Tab.1). In some provinces the values are higher than the average recorded in Italy and Europe. In the 65 goat sampled herds seropositivity was about 9.2% (6 positive herds).

Ten from the ELISA positive samples were found rt-PCR positive. The sequence analysis indicates that all the amplified samples match with BDV genomes and the phylogenetic analysis revealed that all the viruses clustered in the same group classified as BDV-7 previously described in Italy from Lazio and Tuscany isolates.

According to the first analysis SCC appear to have no significant correlation with serological and virus positivity in the screened farms.

Estimated seroprevalence in flock

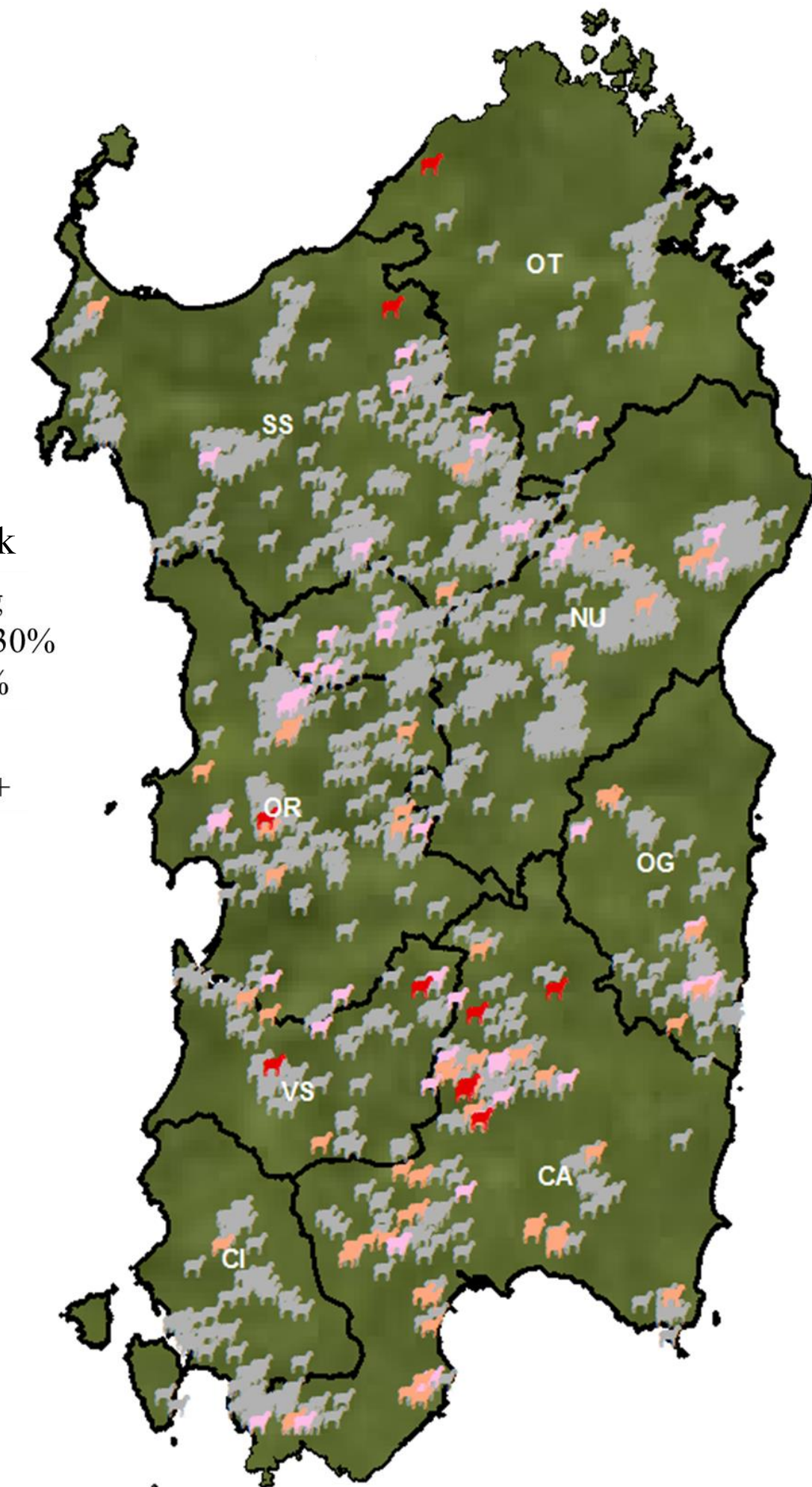
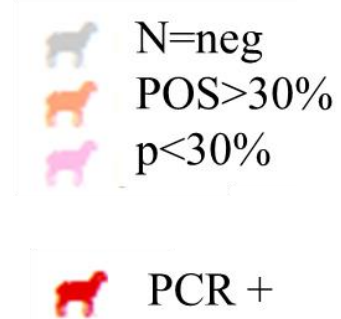
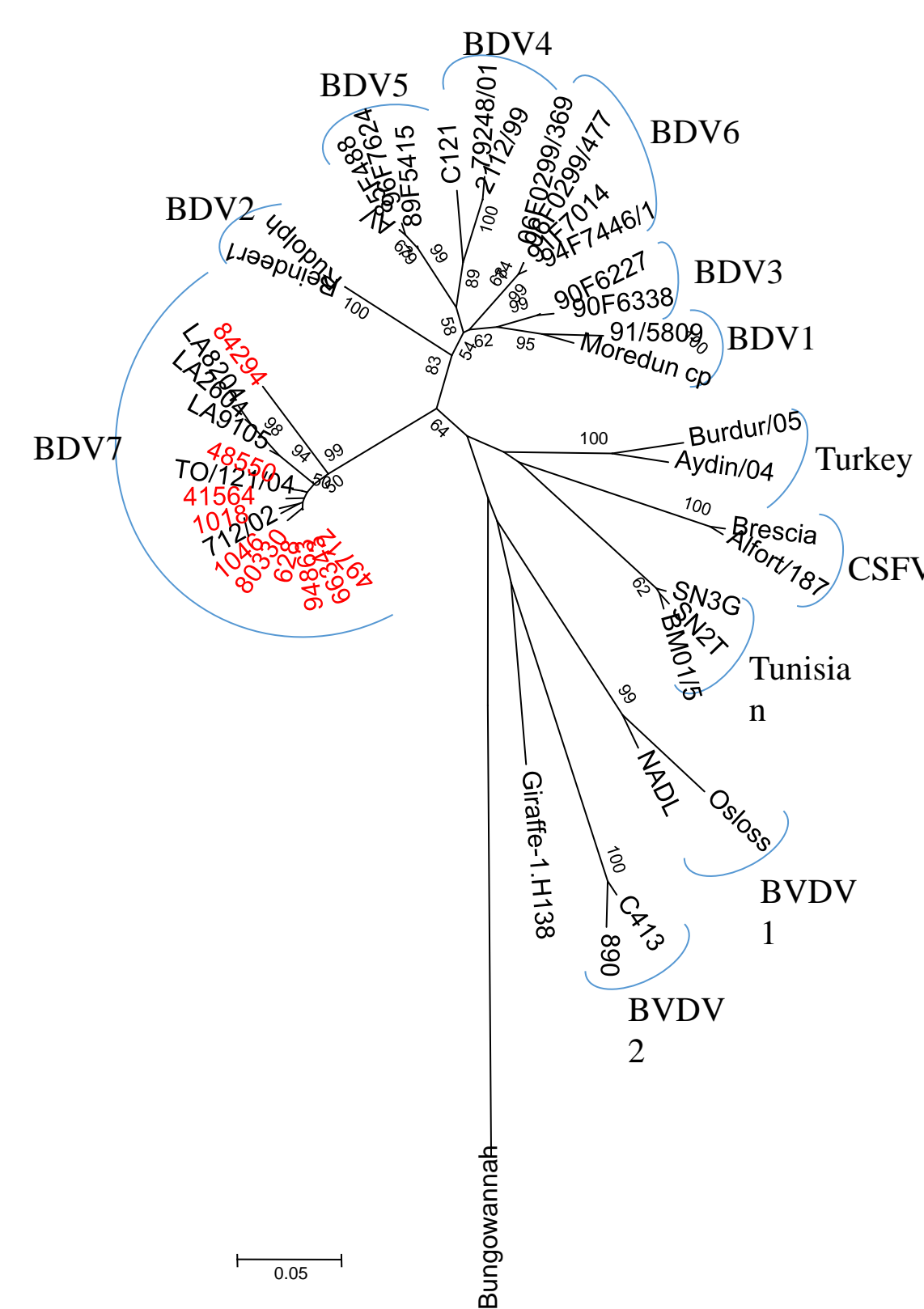


Fig.2 - Distribution of serological and virological positivity in sheep flocks. Seroprevalence in sheep flock was estimated according to AIP (antibody inhibition percentage) values as described for bovine BTM samples. AIP was calculated as: $AIP\% = \frac{ODS\ 450nm\ of\ the\ sample(S)}{ODN\ 450nm\ of\ the\ negative\ control\ (N)} \times 100$.



Phylogenetic tree constructed using 244 nt from the 5'UTR region of the pestivirus sequences found in this study (red) and of 36 reference strains retrieved from GenBank (black). The evolutionary history was inferred using the Neighbor-Joining algorithm with the Kimura 2-parameter method.

The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches. The tree was outgrouped to the sequence of Bungovannah pestivirus (DQ901402).

Evolutionary analyses were conducted in MEGA6. Bar: number of substitutions per site.

CONCLUSIONS

This study represents a snapshot of current distribution of small ruminants Pestiviruses in Sardinia and a genetic characterization of BDV circulating strains. Despite what reported in the rest of Italy, where also BDV-1, BDV-3 and BDV-5 have been isolated, BDV-7 is the only group isolated in Sardinia so far.

Further studies should be led in order to understand the origin, the evolutionary history and the epidemiology of BDV-7 in Sardinia. The role of BDV as an immunosuppressive agent, exacerbating the pathogenicity of co-infecting microorganisms, should also be considered.

REFERENCES

1. Buonavoglia et al., 1994; 2. Graham et al., 2001; 3. Krametter-Froetscher et al., 2007b; 4. Giammarioli et al., 2011; 5. Rosamilia et al., 2014; 6. Giammarioli et al., 2015

ACKNOWLEDGEMENTS

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