Postdoc Fellowships for non-EU researchers

Final Report

Name	Janet HIGUTI
Selection	2012
Host institution	Royal Belgian Institute of natural Sciences, Brussels
Supervisor	Koen MARTENS
Period covered by this report	from 01/08/2013 to 30/11/2013 from 01./06/2014 to 30/11/2014 from 01/10/2015 to 30/11/2015
Title	**A comparative analysis of the biodiversity of Ostracoda (Crustacea) in the Congo River (Africa) and Amazon River (South America) catchments.**

1. Objectives of the Fellowship (1/2 page)

The **strategic objective** of the present postdoc application was to increase the taxonomic expertise on ostracods from two of the largest rivers (and their catchments) in the world, so that meaningful comparisons can be made at the specific level. These comparisons are important to understand not only alpha (local) and gamma (regional) biodiversity, but also beta diversity (change of faunal composition between areas). A clear understanding of such spatial patterns is vital in order to optimise conservation strategies.

In order to achieve this goal, the **operational objectives** are:
(1) Identification of the South American and African species presently in open nomenclature, using literature and collections present at the RBINSc.
(2) Description of new genera and species using facilities of the RBINSc (light and scanning electron microscopes).
(3) Establish if the root systems of the floating water hyacinth, *Eichhornia crassipes*, indigenous in the Amazon and introduced in the Congo (amongst other African water bodies) are populated by local pleuston or if South American ostracod species have been introduced in Africa by the invasive *Eichhornia*.
(4) Comparison of Congolese ostracod communities in indigenous (*Vossia*) and introduced (*Eichhornia*) macrophyte stands
(5) Comparative analysis of ostracod biodiversity within and between the two floodplains/ catchments.

2. Methodology in a nutshell (1/2/ page)

The ostracod collection of the Amazon floodplain used in the present study resulted from a larger CNPq-funded project, which covers four Brazilian floodplains (in addition to the Amazon: Paraná, Araguaia and Pantanal). Two sampling campaigns were conducted in the Amazon floodplain, the first one was done in October 2011 (dry season) and the second one in May 2012 (wet season). More than 20 lakes or lake-like habitats were sampled, mostly for their pleuston communities in *Eichhornia crassipes* stands.

The ostracod collection from the Congo River was effected during the large Congo-expedition in May and June 2010 (Boyekoli Ebala Congo 2010 - http://www.congobiodiv.org/en). More than 50 samples were collected, either in the pleuston of the invasive species *Eichhornia crassipes* or amongst stands of the indigenous *Vossia cuspidata*.

Sampling was done in both rivers using a handnet with a mesh size of c 160 µm. *Eichhornia* roots in both rivers were washed in a bucket (each time three plants) and then cleaned over the handnet. Ostracods in *Vossia* stands were collected directly by moving the handnet through the rooted plants for c 5 minutes. All ostracods were killed by adding 97% ethanol to the wet residual; all samples were washed again in the lab and were transferred to fresh ethanol. Samples are sorted under a binocular microscope. Specimens are dissected with two needles in glycerine. Soft parts are stored in glycerine in sealed slides and are illustrated with *camera lucida*. Valves are stored dry in micropalaeontological slides and are illustrated using Scanning Electron Microscopy.

A parametric analysis of variance (ANOVA) was performed to test for significance of differences in species richness, abundance, diversity and Evenness of ostracods between plants species and between catchments. When the homogeneity assumption required for ANOVA was not fulfilled, a non-parametric Kruskal-Wallis test was used. Analyses of variance were performed in Statistica 7.1 (StatSoft 2005). Rarefaction curves were computed using the BioDiversity Pro Programme (McAleece et al. 1997).

Principal Coordinates Analysis (PCoA) was used to evaluate the (dis)similarity of species composition between different catchments. Differences between the two catchments were tested by ANOVA applied to the scores of PCoA axes. The dispersion homogeneity test (PERMDISP) was performed to test the variability in the ostracods species composition (beta diversity) within Amazon and Congo River catchments. The analyses were performed using the R 3.0.1 software (R Development Core Team 2013) through vegan and permute packages (Oksanen et al. 2013).

3. Results & Discussion (6-8 pages)

(Remark: references are not listed, but can be obtained upon request)

Results

We recorded 25 species of ostracods associated to *E. crassipes* in the Amazon River catchment and 40 species in the Congo River catchment. Of these 40 Congolese species, 31 were found in the invasive *E. crassipes* and 27 ostracod species occurred in the native *V. cuspidata* (Table 1). The most speciose ostracod subfamily was Cypricercinae (8 species in *E. crassipes*) in the Amazon; and Cypridopsinae (9 species in both *E. crassipes* and *V. cuspidata*) and Herpetocypridinae (7 species in *E. crassipes* and 6 species in *V. cuspidata*) in the Congo River catchment. The only ostracod species present in both catchments was *Stenocypris major*, a circumtropical species. Several ostracod species in the Amazon and nearly all species in the Congo River catchment are new to science and remain to be described.

Table 1 Comparisons between ostracods fauna from Amazon (South America) and Congo River catchments (Africa).

Family	Subfamily	AMAZON		CONGO			
		E. crassipes		*V. cuspidata*		*E. crassipes*	
		Genera	Species	Genera	Species	Genera	Species
Cyprididae	Cypricercinae	3	8	1	5	1	4
	Cypridinae	1	2	-	-	-	-
	Cypridopsinae	4	5	2	9	2	9
	Cyprettinae	1	3	-	-	-	-
	Herpetocypridinae	1	1	3	6	3	7
Candonidae	Candoninae	2	3	1	2	1	2
	Cyclocypridinae	1	2	-	-	2	2
Limnocytheridae	Timiriaseviinae	1	1	2	3	3	4
Darwinulidae	Darwinulinae	-	-	2	2	3	3
		14	25	11	27	15	31

The highest diversity and abundance of ostracods were recorded in the Congo River catchment in both aquatic plants species, where richness and Shannon diversity values were higher in the invasive *E. crassipes* (Fig 1a, b, c). However, no significant difference was observed regarding these attributes between *E. crassipes* and *V. cuspidata*. When comparing diversity of ostracods associated with *E. crassipes* between both catchments, significantly higher values of species richness ($p<0.01$) and diversity ($p<0.05$) were found in the invasive plant from the Congo River catchment. The evenness values were similar between Amazon and Congo River catchments (Fig 1d).

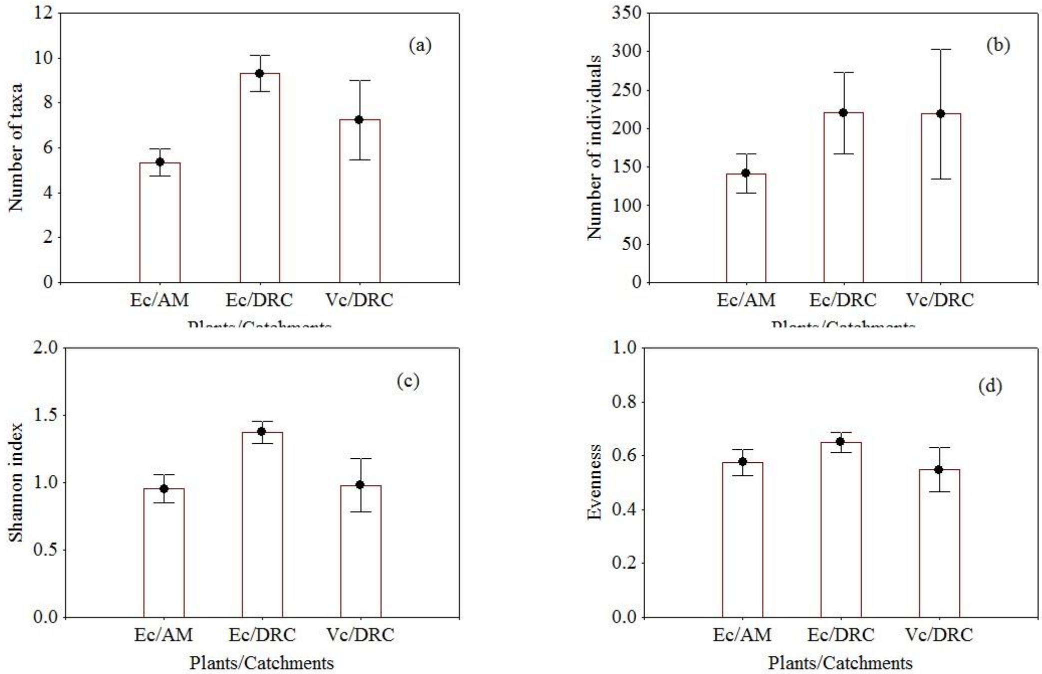


Fig 1 Mean values and standard error of (a) richness, (b) abundance, (c) Shannon diversity and (d) evenness of ostracods community associated with *E. crassipes* (Ec) and *V. cuspidata* (Vc) in Amazon (AM) and Congo River (DRC) catchments.

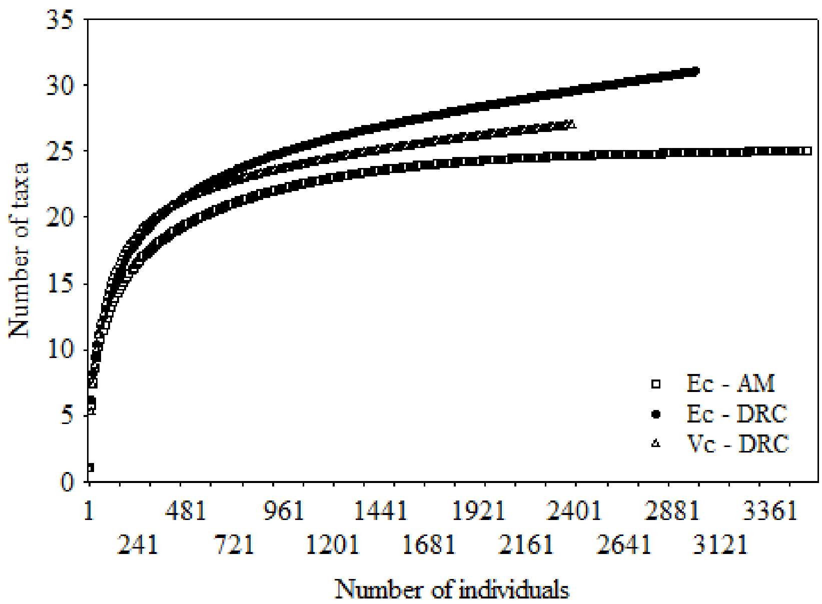


Fig 2 Rarefaction curves of ostracods species associated with different aquatic plants in Amazon (AM) and Congo River catchments (DRC). Ec = *E. crassipes* and Vc = *V. cuspidata*. Square: native *E. crassipes* of the Amazon River catchment, full circle: invasive *E. crassipes* of the Congo River catchment, triangle: native *V. cuspidata* of the Congo River catchment.

Rarefaction curves indicated higher number of ostracods species in the Congo River catchment, especially on the invasive *E. crassipes*, for similar numbers of individuals. The richness reached an asymptote in the *E. crassipes* samples from the Amazon River catchment, while it is still increasing in the *E. crassipes* and *V. cuspidata* samples of the Congo River catchment (Fig 2), meaning that with more sampling most likely still more species could be found there.

The result of the Principal Coordinates Analysis (PCoA), used to evaluate the (dis)similarity between different catchments, showed significant differences in species composition, assessed for the scores of PCoA, axis 1 ($F = 170.98$; $p < 0.00$) and axis 2 ($F = 5.39$; $p < 0.05$). The dispersion homogeneity test (PERMDISP) showed no significant differences ($F = 0.31$; $p = 0.59$) in the variability of ostracod species composition (beta diversity) within Amazon and Congo River catchments. The highest average distance to the centroid was recorded in the Amazon River catchment (0.58) compared to the Congo River catchment (0.56) (Fig 3).

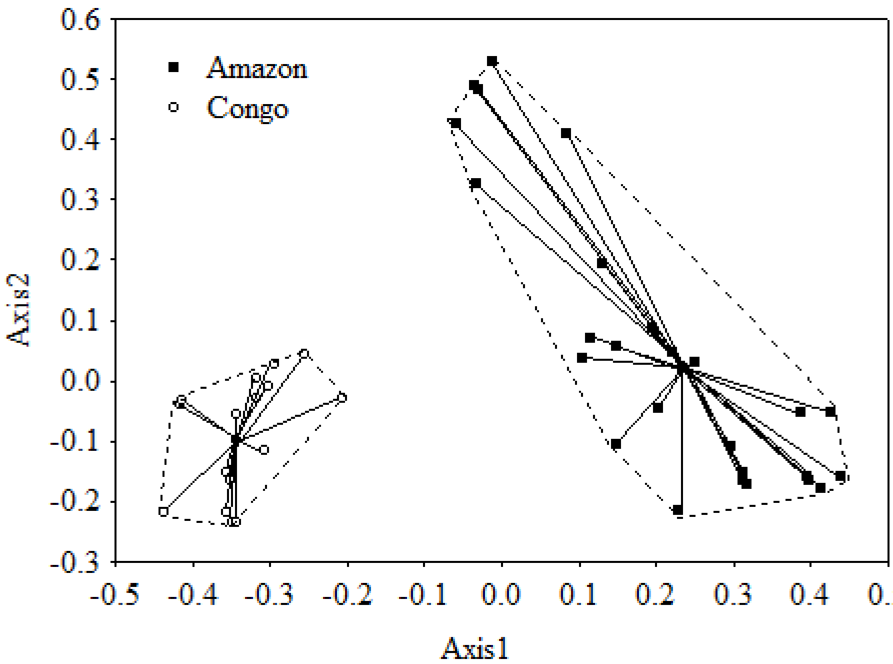


Fig 3 Multivariate permutation analysis diagram showing the variability in ostracods species composition associated with *E. crassipes* between different catchments. Full square: Amazon River catchment, circle: Congo River catchment.

Discussion

Composition of ostracod communities

Ostracods are amongst the most abundant invertebrates associated with macrophytes and are themselves a group that have a high diversity of species (Thomaz et al. 2008; Higuti et al. 2007, 2010; Liberto et al. 2012; Mazzini et al. 2014; Matsuda et al. 2015).

The Cyprididae are the most speciose ostracod family in the Amazon and Congo River catchments. This is in line with the fact that this family comprises c 50% of all known Recent non-marine ostracod species and more than 80% of all species in most tropical regions (Martens 1998). The patterns of species composition are different between both catchments: Cypricercinae (32% of species) and Cypridopsinae (20%) are the most dominant subfamilies in the Amazon River catchment, Cypridopsinae (30%) and Herpetocypridinae (20%) in the Congo River catchment. These findings are in agreement with those of Martens (1998), who compared the diversity of non-marine Ostracoda from Africa and South America.

The comparison of the ostracod communities between the two catchments clearly shows that the pleuston in the Amazon River catchment only contained South American species, while those in the Congo River catchment only contained Congolese ostracod species, or in any case did not have any identifiable South American (or other non-African) ostracod species, with *Stenocypris major* being the only shared, circumtropical, ostracod species. The invasive *E. crassipes* therefore did not act as a "Noah's Arc" by transporting also invasive ostracod species into that part of the Congo River catchment. This can be either because the invasive plant was introduced as seeds or propagules, in which case no pleuston was associated with it yet, or because the invasive pleuston in adult plants failed to establish viable communities in the new environment.

Comparison between ostracod communities in native (Amazon) and invasive (Congo) Eichhornia crassipes

Our results showed higher ostracod diversity and abundance in the invasive *E. crassipes* in the Congo River catchment than in the native *E. crassipes* specimens from the Amazon River catchment. This is a rather unexpected result, as one could predict to have a greater diversity of ostracod species in *E. crassipes* of the Amazon River catchment, where this plant is native and where ostracod species are expected to be better adapted to life in the root system of this (and other) floating plants. The fact that the collections were sampled in a small part of the Amazon floodplain relatively near to the city of Manaus might be part of the reason. However, the rarefaction analyses showed that the cumulative curve of ostracod species in *E. crassipes* from the Amazon River catchment had reached the maximum plateau with our collections. The results are thus representative for at least that part of the Amazon River catchment. Moreover, size of root systems in this macrophyte species can vary considerably and is known to depend at least in part on water chemistry (Kobayashi et al. 2008). Observations showed that the root systems of this plant in the Congo River catchment samples were much smaller than those from the Amazon River catchment, down to less than half the volume (results not shown). This makes the higher values of richness, diversity and abundance of the ostracod communities in the Congolese samples even more surprising.

The present results indicate that invasive species such as *E. crassipes* do not necessarily always have a fully negative effect on the invaded communities. Invasive *E. crassipes* root systems obviously offer novel substrates for native animals, such as ostracods, that can adapt to life in pleuston. However, this is clearly only one side of a broader picture, where other native communities such as zooplankton or whole community levels might be negatively impacted.

*Comparison between ostracod communities in native (*Vossia cuspidata*) and invasive (*E. crassipes*) plant species in the Congo River catchment.*

We observed that the ostracod communities associated with an invasive plant species, *E. crassipes*, have a higher richness, diversity and abundance than those associated to a native plant species, *V. cuspidata*, in the Congo River catchment. This is a clear example of an invasive species physically facilitating communities of native species (see review in Rodriguez (2006)).

Also Mormul et al. (2010) observed higher values of these attributes of ostracod communities in an invasive plant, *Hydrilla verticillata* when compared to a native macrophyte, *Egeria najas*, in the Paraná River floodplain, although these differences were not significant. Howevever, cumulative curves did indicate higher diversity of ostracod species on the invasive *H.*

verticillata. The invasive plants might thus provide favorable habitats for native ostracod communities. The experiment by Mormul et al. (2010) was performed to test the effect of an invasive plant on the ostracod communities, using two plants with similar architecture (as measured in fractal heterogeneity). In the present case, *Eichhornia crassipes* and *Vossia cuspidata* do not have similar architecture: *Eichhornia* is a floating plant with normally dense root systems with high heterogeneity, whereas *Vossia* is a grass-like rooted plant, with low individual heterogeneity but occurring in dense stands. This density of the macrophyte stands will to a certain degree compensate for the lower individual heterogeneity.

Figueredo et al. (2015) conducted an experiment using a native (*E. najas*) and an invasive (*H. verticillata)* macrophyte species to test the preference of a native fish prey species for either macrophyte as a refuge in the presence of an invasive voracious predator. It appeared that the prey fish did not show preference for one plant over the other, and they concluded that habitat structure affects choice more than evolutionary history between fish and macrophytes and this is in line with our results.

The effects of invasive plants on aquatic communities, however, is more complex and depends on more factors than on heterogeneity of the habitat only. Villamagna and Murphy (2010) showed that abundance and diversity of aquatic invertebrates generally increase in response to increased habitat heterogeneity and structural complexity provided by *E. crassipes*, but can also decrease due to decreased planktonic algae (food) availability. Stiers et al. (2011) compared macrophytes and macroinvertebrates communities of 32 Belgium ponds, of which 22 included invasive plants. Negative impact on native plants and macroinvertebrates was recorded for three invasive plants, reducing the richness of both communities. Schultz and Dibble (2012) summarized the role of invasive plants on freshwater fish and macroinvertebrate communities. They found that positive effects of invasive macrophytes on fish and macroinvertebrate communities were associated with characteristics held in common with native macrophytes such as photosynthesis, increasing habitat heterogeneity, and stabilizing substrate. The authors found that three other traits of invasive plants can largely be responsible for negative effects on fish and macroinvertebrate communities: increased growth rate, allelopathic chemical production, and phenotypic plasticity that allow for greater adaptation to environmental conditions and resource utilization than native species.

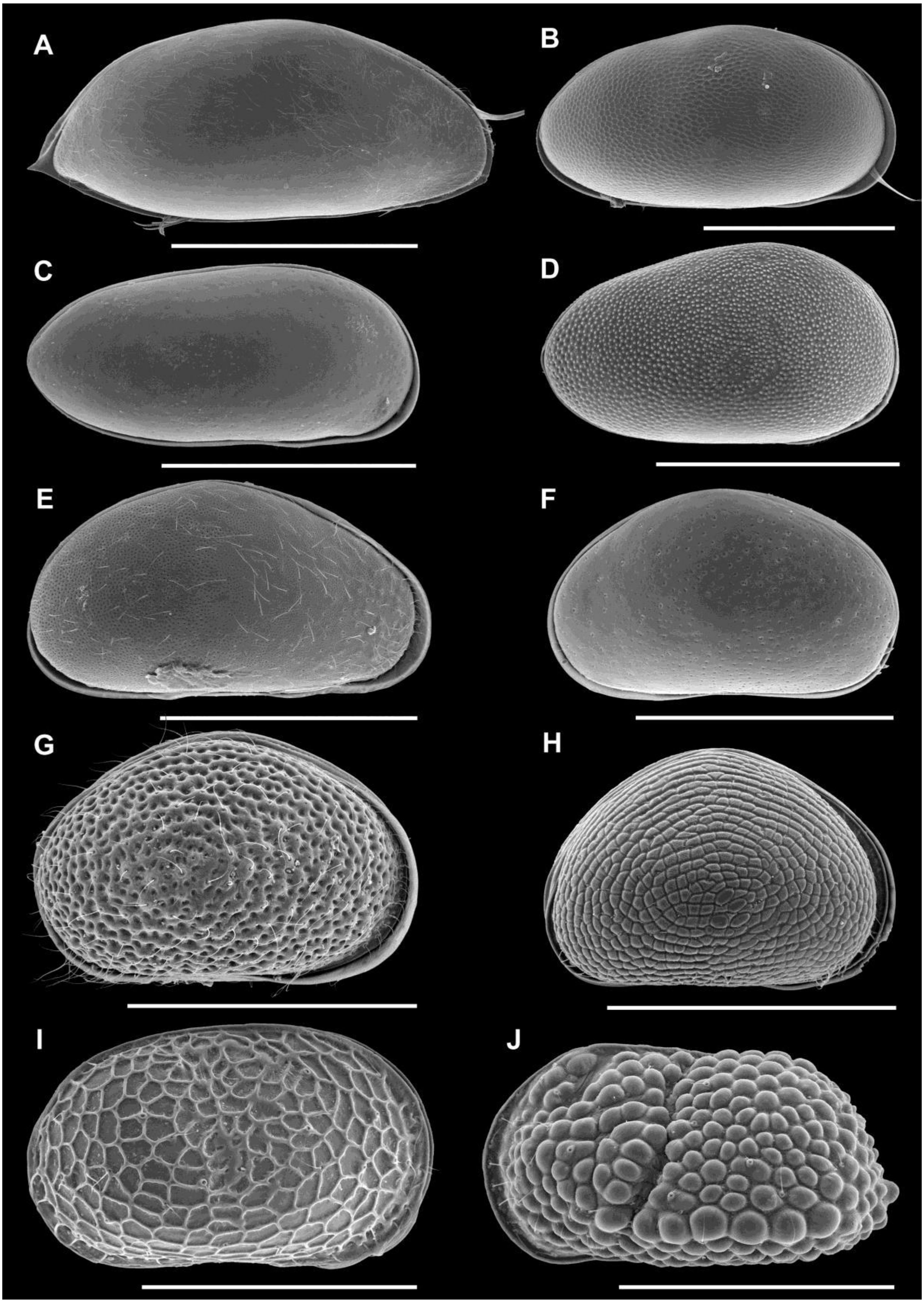
Conclusions

Ostracod communities in Congo and Amazon River catchments are highly dissimilar, indicating that the invasive *Eichhornia crassipes* root systems in the Congo River catchment were colonized by African ostracods, and that the arrival of invasive *E. crassipes* did not bring along successful invasions of South American ostracod species, nor species from other parts of the world from where the plants may have been introduced into the Congo basin. Also, richness, diversity and abundance of ostracod communities in the invasive Congolese plants are higher than in the native Amazonian plants. Finally, richness, diversity and abundance is also higher in the invasive *E. crassipes* than in the native *Vossia cuspidata* in the Congo River catchment, but this could in part be owing to lower complexity of the native plant. It appears that local African ostracod faunas have adapted to exploit the habitat opportunities presented by the floating invasive *E. crassipes*.

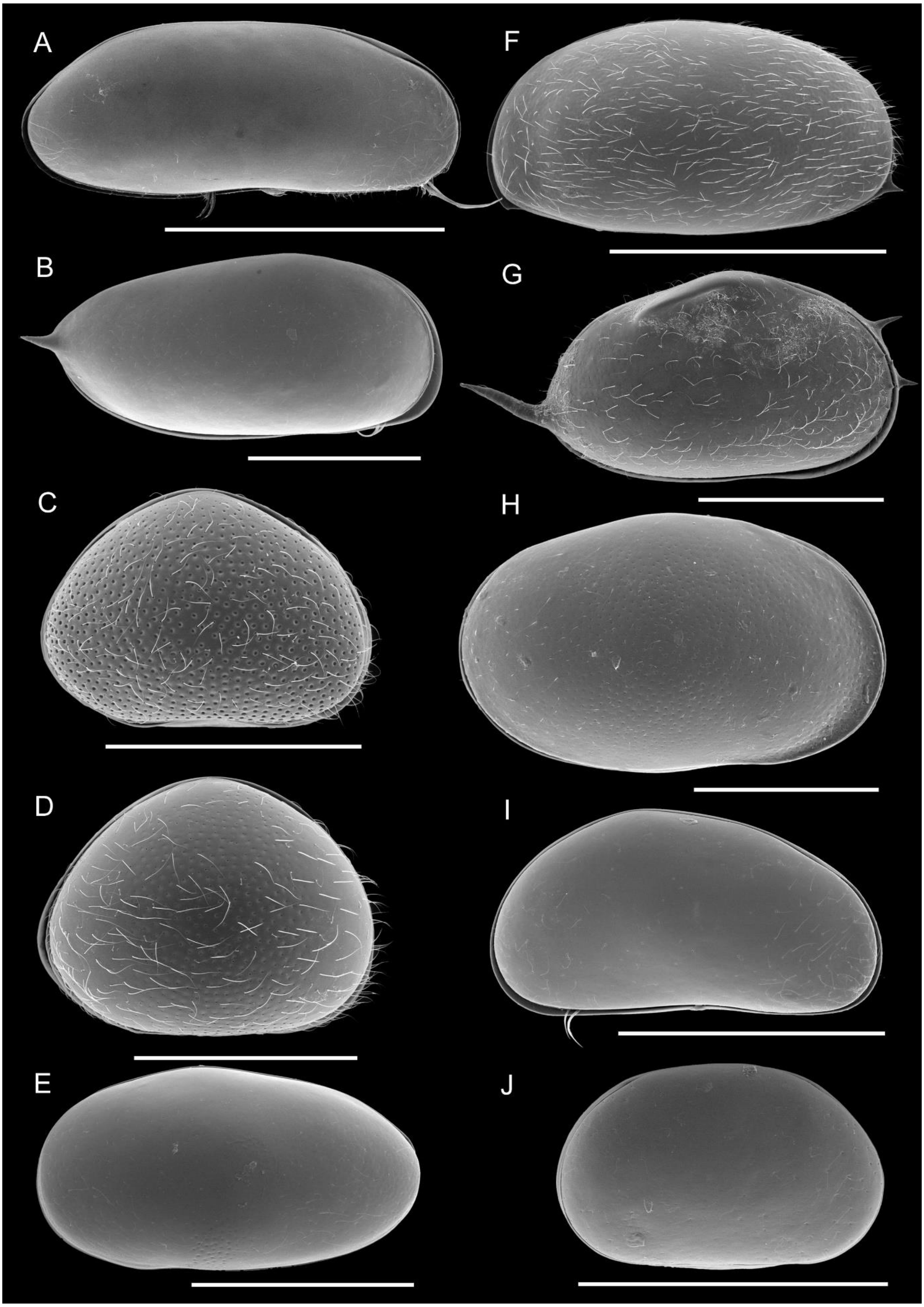
Meeting the Operational Objectives of the fellowship (see above sub 1)

- Operational objectives 1 and 3-5 have been met in full and the results are written down in the (accepted) paper in the journal Biological Invasions, to be published in 2016 (see below)
- Operational Objective 2 is partly fulfilled and several new taxa have already been described (see below). However, most of the taxa found in the Congo River turned out to be new to science and there was simply too little time to deal with such unexpected wealth of undescribed biodiversity. In addition, a large part of the material consisted of single

specimens or decalcified samples (because of the low pH of the Congo River water) and such material is insufficient to be used for species description. However, the collaboration is ongoing and new taxa resulting from this fellowship will be continue to be described in the future (see below sub 4).



Appendix 1. Examples of ostracods from Congo River, illustrated with Scanning Electron Microscopy. A. *Acocypris capillata*; B, C, D. *Strandesia*; E, F. *Cypridopsis*; G, H. *Zonocypris*; I, J. *Gomphocythere*. B-J all new species. Scale bars: A=1mm; B, D= 500µm; C= 400 µm; E-J= 300 µm.



Appendix 2. Examples of ostracods from Amazon floodplain. illustrated with Scanning Electron Microscopy. A: *Stenocypris major*; B: *Cypricercus centrura*; C: *Cypretta* sp.5 n.sp.; D: *Cypretta* sp.6 n.sp.; E: *Strandesia lansactohai*; F: *S. psittacea*; G: *S. trispinosa*; H: *S. velhoi*; I: *Pseudocandona agostinhoi*; J: *Physocypria* sp. 1 n.sp.. Scales bars: A e F = 1000 µm; B, E, G, H, I e J = 500 µm; C e D = 400 µm.

4. Perspectives for future collaboration between units (1 page)

- The present Fellowship was conducted in the framework of a bilateral agreement: "AGREEMENT FOR COOPERATION BETWEEN UNIVERSIDADE ESTADUAL DE MARINGÁ, BRAZIL AND THE ROYAL BELGIAN INSTITUTE OF NATURAL SCIENCES, BELGIUM" which was signed by both parties in May 2011.

- Collaborations between both institutions has been ongoing in the form of several projects, of which the present Fellowship (funded by Belspo) and the project SISBIOTA (funded by CNPq – this project provided the material from Amazon) are but two examples.

- The present report is jointly written during a visit of K. Martens to Nupelia (Maringá) 7-30/1/2016, during which further steps in the valuation of the joint work, especially on Amazon and Congo, but also on other Brazilian floodplains is discussed.

- A synthetic paper on all results from the SISBIOTA project is being written by all participants; K. Martens plays a role in producing what will be a high level joint publication. Temporary title: **Floods as major drivers of metacommunity structure across South American floodplains.**

- Several joint student projects are presently running, mostly on the ecology of ostracods from Brazilian floodplains, with resulting from SISBIOTA but also from other CNPq projects, such as Long-Term Ecological Research - LTER in Paraná floodplain, and "Upper Paraná River: longitudinal gradient of environmental variables and aquatic communities in the last stretch free of dam between Porto Primavera hydroelectric and Itaipu reservoir", etc.

- Koen Martens has collaborated with Janet Higuti to give a post-graduation course on "Taxonomy and Ecology of non-marine Ostracoda" (18-22/1/2016). It is hoped to continue this course at an annual or biannual frequency.

- Several new joint proposals for CNPq projects are presently being prepared, to ensure that future initiatives can also be funded.

5. Valorisation/Diffusion (including Publications, Conferences, Seminars, Missions abroad...

Popular

- The postdoc of Janet Higuti was described in a full article in Science Connection (nr 40, 2013) by Bogdan Van Doninck: Mobilité en action: une chercheuse brésiliene à Bruxelles.

Posters

- HIGUTI, J. & K. MARTENS, 2014. Ostracoda (Crustacea) Fauna of Congo River (Africa) and Amazon River (Brazil) Catchments (Poster). Kisangani, June 2014.

- MARTENS, K & J. HIGUTI, 2015. Comparison of ostracod (Crustacea) pleuston in *Eichhornia crassipes* (Mart.) from the Congo (Africa) and Amazon (South America) rivers. Poster presentation (by both JH and KM in person) at the XV Congresso Brasileiro de Limnologia, Maringá (Brazil), July, 2015.

Papers

- HIGUTI, J**.** & K. MARTENS 2014. Five new species of the Candoninae (Crustacea, Ostracoda) from the alluvial valley of the Upper Paraná River (Brazil, South America). European Journal of Taxonomy 106: 1-36. (IF 2014 = 1.312). (Remark: also occurring in Amazon).

- HIGUTI, J. & K. MARTENS 2016. Invasive South American floating plants are a successful substrate for native Central African pleuston. Biological Invasions (accepted). (IF 2014= 2.586).

Missions

- Janet Higuti has divided het 12 months postdoc in Brussels over three periods: 2013 (4 months), 2014 (6 months) and 2015 (2 months). Travel of the last two trips was funded outside of the present Belspo Fellowship.

- Koen Martens has made 3 visits to Nupelia (Maringá) during the period 2013-2015: 9-30/3/2014; 28.1-20.2/2015 and 29/6-21//7/2015. During these visits, Amazon and Congo River material was identified and results analysed and discussed. All of these trips were funded outside of the present Belspo Fellowship.

6. Skills/Added value transferred to home institution abroad (1/2 page)

- Drafting collection schemes of ostracods in vegetation, including replication schedules and avoidance of pseudo-replication;

- Sample treatment for both ecological and molecular analyses, sorting living and dead ostracods, museological good practices for long term preservation and storage of samples and specimens : use of proper vials and preservation material, long-term labelling, databasing of data linked to sample- and specimen collections;

- Ostracod identification using original publications, creation of a regional atlas for identification based on specimens, labelling new species with temporary monikers, …

- Illustration of valves with Scanning Electron Microscopy (preparation of specimens on stubs, re-distribution of valves in properly labelled micropalaeontological slides, use of valves to prepare plates for atlas or for publications;

- Dissection of ostracod soft parts, preparation of permanent slides, labelling, storage and electronic registration;

- Illustration of soft parts (limbs, hemipenes, ..) with camera lucida, preparation of plates for atlas and for publications;

- Basic principles of taxonomy, nomenclature and phylogeny;

- Preparation of full descriptions of new species and genera; preparation if type materials for deposition in official museum collections, registration.