The main objective of the proposal presented was to progress knowledge on the evolution of Tardigrada, using all the information we would be able to generate: morphological, molecular, biological, etc. Once we would have a reasonably stable phylogeny, we would study the evolution of different characteristics in tardigrades e.g., reproductive modes, their distribution through different habitats, claws and buccopharyngeal apparatus morphology, substances from cryptobiosis and cryptobiosis as a process itself, etc. These results would be useful both in invertebrate and within Tardigrada evolution, and in biomedicine (within the evolutionary biomedicine framework). One of the main objectives was to include more taxa and genes (mainly those used in other invertebrate analyses, so our results could be used in other invertebrate evolution studies) in phylogenetic analyses. Besides the above, we have tried to study and analyse different morphological characters (examining Tardigrada collection from the Museum of Zoology at the University of Copenhagen), to complete previous information, so that complementary information will be available for further analyses. Finally, we would perform phylogenetic comparative analyses to study the evolution of certain interesting characters within tardigrades.

**Molecularly**, we have advanced in protocols from its more basic steps to primers useful for tardigrades, and we have settled the bases of general DNA protocols for tardigrades in accordance with other Tardigrada group working on molecular, Prof. R. Bertolani´s team. Moreover, we have increased greatly in taxa and genes (majority of 28S RNA information available at present days) for Eutardigrada analyses comparing with what was published either in journals or in GenBank. A total of 153 sequences have been obtained (DNA extraction, DNA amplifications of specific fragments, cleaning those amplifications and sequencing them), 93 from class Eutardigrada and 60 from class Heterotardigrada, belonging to 45 species (27 Eutardigrada and 18 Heterotardigrada) and 88 specimens (40 were eutardigrades and 48 heterotardigrades). For each specimen I have sequenced a 700 bp fragment of the 18S RNA and a 1500 bp fragment of the 28S RNA. In the analyses about **Eutardigrada** genera, 15 out of 27 species sequenced have not been sequenced before: *Doryphoribius macrodon* (Greenland), *D. zyxiglobus* (Australia), *Hypsibius dujardini* (Greenland), *Ramazzottius cataphractus* (Greenland), *Diphascon prorsirostre* (Denmark), *Dactylobiotus ambiguus* and *D. octavi* (Greenland), *Murrayon pullari* (Greenland), *Adorybiotus granulatus* (Greenland), *M. echinogenitus* (Faroe Island), *M. grandis* (Greenland), *M. harsmworthi* (Faroe Island), *M. islandicus* (Greenland), *M. liviae* (Chile), and *Milnesium eurystomum* (Spain). Species sequenced before for 18S RNA but for which we have included geographical variability are: *Ramazzottius oberhaeuseri* (from Sweden and Greenland), *Milnesium tardigradum* (Greenland, Disko Island and Norway), and *Isohypsibius prosostomus* (Egypt and Denmark). For majority of Eutardigrada sequences I have sequenced 28S RNA information not available before. There are 11 out of 18 **Heterotardigrada** species not sequenced before, and are: *Bryochoerus intermedius* (Greenland), *Bryodelphax aaseae* n. sp. (Easter Island, Chile), *Proechiniscus hanneae* (Disko Island, Greenland), *Pseudechiniscus novaezelindae* (Australia), *P. suillus* (Norway), *Echiniscus bigranulatus* (Chile), *E. blumi* (Chile and Greenland), *E. merokensis suecica* (Greenland), *E. oihonnae* (Normay), *E. spiniger* (Sweden), and *E. wendti* (Greenland). New, universal and specifically designed, primers for both nuclear genetic markers (18S RNA and 28S RNA) have been tried successfully. Almost all 28S RNA information I have created is new in GenBank data base. Sequences have been check and are now ready in a matrix for phylogenetic analyses.

**Morphological** analyses of specimens from the Prof. R. M. Kristensen collection and identification of individuals for subsequent DNA extraction have required the use of an optical microscope. I have had the opportunity to study species from three families from class Eutardigrada I have not observed before (*Calohypsibius ornatus*, from family Calohypsibiidae; *Bertolanius* from Amphibolidae; and *Eohypsibius* and *Microhypsibius* from Microhypsibiidae), analysing diverse morphological structures, making digital photographies of them, and recording them in a data base. Besides, I have studied many genera from Eutardigrada and Heterotardigrada I had not observed before: *Pseudobiotus, Richtersius and Xerobiotus* from class Eutardigrada, *Echiniscoides, Halobiotus, Briodelphax,*

*Bryochoerus, Proechiniscus,* and *Testechiniscus* from class Heterotardigrada, and several species from genera *Echiniscus* and *Pseudechiniscus* (Echiniscidae, Echiniscoidea, Heterotardigrada). Besides, a study of tardigrades from Morocco belonging to new material, donated by Drs. J. Hortal Muñóz and Ana Santos from the Imperial College of London (U.K.), have required to fixed in Carnoy and mount in Faure liquid for optical microscope preparations: 108 heterotardigrades, 356 eutardigrades and 58 eggs, now deposited in the invertebrate collection of the Museum of Zoology at the University of Copenhagen. These specimens have been observed and identified in a first round, but a second round for all of them and more photos will be recorded. Revisions of Morocco material from Prof. R. M. Kristensen collection and comparisons with new material are final steps to this task.

After collecting all molecular and morphological information, **phylogenetic analyses** with molecular information (18S RNA and 28S RNA information analyzed together and separated) would be performed comparing a more complete matrix in genetic information with both genetic markers (18S RNA and 28S RNA) but only with taxa sequenced in this project (more complete genetic information since GenBank information on Tardigrada is focused on 18S RNA), and with all taxa available (from our study but also from GenBank) but an incomplete matrix in genetic information (high number of missing data mainly on 28S RNA marker). These new information together or not with previous data would produce a more stable and robust phylogenetic hypothesis about tardigrade´s evolution, since we have included at the end taxa from both classes (Eutardigrada and Heterotardigrada). Morphological information from references and the one I have obtained from direct observations on material from collections, but also from specimens dedicated to DNA extractions, would be indispensable to interpret phylogenies achieved as well as to perform comparative phylogenetic analyses, and discuss about evolution of those morphological characters. Morphological experience that I have acquired, through the study of different morphologies, would be also useful to carry out phylogenetic analyses of Eutardigrada genera using only morphological information, and so to propose a phylogenetic hypothesis to discuss about which morphological characteristics have influenced eutardigrade evolution. Phylogenetic result from molecular and morphological analyses would be compared and combined to understand the evolution within this phylum. Phylogenies obtained will be more stable and results better supported because of increase in information (morphological and molecular) we have provided. Analyses with *Echiniscus* species including several other genera of the family (Echiniscidae) will be one the few studies about heterotardigrades using molecular information. This will be a big step within the class since little previous information is available. Besides, the topic we are studying within this genera, its monophyletic status, open the discussion about the necessity to check phylogenetic relationships at higher (more popular and easily published) but also at lower (harder to publish since it concern to fewer researchers) taxonomic levels since the need to understand both taxonomic levels in terms to clarify evolution of the whole phylum, and so, animal´s evolution.

In the same way that Eutardigrada phylogeny will be useful (and is needed) for studies among animal phyla, determining monophyletic status of Heterotardigrada taxa it is the preliminary and necessary step to future phyletic discussions among heterotardigrades, but also within Tardigrada and among phyla (which have not had into account heterotardigrades but eutardigrades in their analyses; molecular differences observed in these project between the two classes highlight the necessity to include Heterotardigrada in phylogenetic analyses among animal´s phyla). In a closer look, all new sequences obtained in this project will be useful for tardigradologist who study molecular aspects of this animals, since a more complete data base of sequences is now available, for example to design new and specific primers. Results obtained from all these analyses will be helpful not only among tardigradologists, but also for researches in invertebrate evolution, and, as a consequence, in understanding life patterns and processes. An increase of knowledge within tardigrades will probably solve, or at least will shed some light, to understand Ecdysozoan´s (animals with molting stages) evolution, since this is, very likely, a key group in the evolutionary framework of animals. Furthermore, an innovative field such as biomedicine, as well as those research lines which investigate cryptobiosis and their possible future applications, need from this basic evolutionary knowledge to progress and to