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Cryptic polychaete diversity: a review

ARNE NYGREN

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This review summarises the current knowledge on cryptic species of polychaetes, one of the most dominant taxa in marine communities, and gives a brief overview of the different methods that has been used for their disclosure. Cryptic species constitute an important part of biodiversity and they are common among all kinds of polychaetes, with different life history traits, and may have sympatric as well as allopatric distributions. It is emphasised that cryptic species must not be neglected for a variety of reasons, and even though the magnitude of cryptic species is largely unknown the available data on polychaetes clearly demonstrate that morphology alone seriously underestimate the number of species. It is suggested that future research should focus on appropriately designed case-studies using combined approaches, as well as on large-scale whole sample analyses using next-generation sequencing in order to be able to answer the many questions that still remains.

Corresponding author: *Arne Nygren, Systematics and Biodiversity, University of Gothenburg, Box 463, SE-405 30 Gothenburg, Sweden. E-mail: arne.nygren@bioenv.gu.se*

Introduction

It has been argued that the marine environment alongside the tropical rainforest should be the biomes that harbour the greatest part of cryptic biodiversity on earth (Bickford *et al.* 2007). Whether this is a correct prediction or not (Pfenninger & Schwenk 2007; Trontelj & Fiser 2009), there is no doubt that the sea swarms with cryptic species, and they are found in all major taxonomic groups (Knowlton 1993, 2000), polychaetes are no exception. Cryptic species are two (or more) morphologically similar species that erroneously have been classified as a single one (Bickford *et al.* 2007). Cryptic is a relative concept, and it is not uncommon that cryptic species, once revealed by other information such as molecular data, are found to have morphological differences (Sáez & Lozano 2005). Such species are instead referred to as pseudocryptic, but the line between cryptic and pseudocryptic is not sharp. With this said, what are the reasons for this ubiquitous presence of cryptisism in the sea? There are several possible explanations.

Our limited access to the marine environment prevents us from properly assessing species boundaries in the first place. Traditionally, polychaete taxonomists have worked with preserved specimens that have been collected during expeditions where the material has been sieved, put into jars together with other organisms, and subsequently killed and preserved unanaesthetised with formalin or alcohol. For soft-bodied animals like polychaetes, this is not an ideal method since many specimens loose their appendages and are fragmented. Potentially valuable and species-specific characters such as colouration are also usually lost. Second, chemical cues are more important than visual signals for mate recognition for many marine organisms (Knowlton 1993; Sutton *et al.* 2005), and changes in the mate recognition chemistry do not necessarily lead to changes in the species phenotype. Finally, many marine taxonomists have been trained in an over-conservative tradition (Klautau *et al.* 1999), where large distribution areas as well as large intraspecific variation have been the accepted norm. Large distribution areas is not impossible per se in the marine environment, since many species have potential for long distance dispersal via planktonic larvae, but to what extent this is realised is poorly known (Knowlton & Keller 1986; Hellberg 2009).

Why are cryptic species important?

Cryptic species are important because of their commonality, and they are routinely found in genetic surveys, also in well-known taxa in well-studied areas (e.g. Bleidorn *et al.* 2006; Jolly *et al.* 2006; Nygren & Pleijel 2011). Since these investigations merely concern a small fraction of all morphospecies of polychaetes, and since large, comprehensive genetic studies on species boundaries in polychaetes are rare, we are probably seeing the tip of the iceberg (Knowlton 1993).

Considering their ubiquitousness cryptic species cannot be neglected, because biological knowledge is often based on studies on restricted populations, which then are referred to by a species name. Even though cryptic species are morphologically similar they may not share the same biological characteristics, and indeed, the opposite often seems to be the case. Cryptic species may differ in reproductive biology (Hauenschild 1951; Smith 1958; Grassle & Grassle 1976; Ekaratne *et al.* 1982; Pfannenstiel *et al.* 1987; Fong & Garthwaite 1994; Sato 1999; Schulze *et al.* 2000; Kruse & Reise 2003; Kruse *et al.* 2003; Sato & Nakashima 2003; Lewis & Karageorgopoulos 2008; Paxton & Åkesson 2010), life history characteristics (Grassle & Grassle 1976; Rice & Simon 1980; Wilson 1983; Mustaquim 1986; Westheide & Rieger 1987; Manchenko & Radashevsky 1993, 2002; Bonse *et al.* 1996; Bastrop *et al.* 1998; Sato 1999; Kikuchi & Yasuda 2006; Lewis & Karageorgopoulos 2008), feeding biology (Wiklund *et al.* 2009a), habitat and depth preferences (Bellan & Lagardère 1971; Paxton 1979; Ekaratne *et al.* 1982; Manchenko & Radashevsky 2002; Kruse & Reise 2003; Nygren *et al.* 2005, 2010; Lewis & Karageorgopoulos 2008; Wiklund *et al.* 2009a; Luttikhuizen & Dekker 2010; Schüller 2011), tolerance to sulphid and anoxia (Gamenick *et al.* 1998; Kruse *et al.* 2004), as well as temperature (Weinberg *et al.* 1990; Blank *et al.* 2006) and salinity preferences (Maltagliati *et al.* 2000, 2001). Cryptic species are also important because several of these complexes are used as bioindicators in environmental monitoring (Grassle & Grassle 1976; Pearson & Rosenberg 1978; Rice & Simon 1980; Durou *et al.* 2007), or in ecotoxicological and bioaccumulation studies (Reish & Stephens 1969; Åkesson 1970, 1983; Reish 1985; Ozoh 1992; Méndez & Páez-Osuna 1998; Virgilio *et al.* 2005; Burlinson & Lawrence 2007; Vázquez-Núñez *et al.* 2007; Dean 2008; Blake *et al.* 2009). The results from such bioassays may not be comparable if different species has been used among those studies, and the results from a single study may also be confounded if more than one species in a cryptic complex was used (Åkesson 1983). Knowledge of and proper identification of cryptic species may be vital for the detection and understanding of invasive species (Zettler *et al.* 2002; Bastrop & Blank 2006). In the studies of the invasive *Marenzelleria* into the Baltic Sea (Bastrop *et al.* 1995, 1998; Röhner *et al.* 1996; Jürss *et al.* 1999; Bastrop & Blank 2006; Blank & Bastrop 2009), no less than three different cryptic species were possible to identify, with potentially different effects on the indigenous fauna.

Whereas the *Marenzelleria* species likely have been introduced via ballast water, another possible source for bioinvasive species is the importation of live bait used in sport fishing or as biomass for feeding finfish and crustaceans (Costa *et al.* 2006). The transportation of live bait around the globe and its associated problems are of course not restricted to cryptic species, but the incorrect assumption that a widely distributed species may be imported without any risk, as long as it is the same nominal species, may lead us astray if cryptic species are involved (Lewis & Karageorgopoulos 2008). Knowledge of cryptic species is also important for the aquaculture industry itself since the different species in a cryptic complex most likely have different biology, competitive ability and pathogen tolerance. There are several species in the cryptic *Perineris nuntia*-group, and in the *Marphysa sanguinea*-complex that are used in this trade and correct identification may be vital for proper management (Glasby & Hsieh 2006; Lewis & Karageorgopoulos 2008).

Without taking cryptic species into account, our understanding of biogeographical patterns will be severely limited. There are many examples of widely distributed polychaete species, at different scales, that has been found to consist of two or several species with more restricted geographic distribution (Guérin & Kerambrun 1984; Weinberg *et al.* 1990; Fong & Garthwaite 1994; Bonse *et al.* 1996; Schmidt & Westheide 1998, 1999, 2000; Scaps *et al.* 2000; Schulze *et al.* 2000; Westheide & Hass-Cordes 2001; Rouabah & Scaps 2003; Westheide & Schmidt 2003; Jolly *et al.* 2005, 2006; Bleidorn *et al.* 2006; Lewis & Karageorgopoulos 2008; Rice *et al.* 2008; Blake 2009; Halt *et al.* 2009; Meißner & Blank 2009; Nygren *et al.* 2009; Wiklund *et al.* 2009a; Barroso *et al.* 2010; Carr *et al.* 2011; Nygren & Pleijel 2011; Borda *et al.* 2013). Furthermore, we should not neglect that the sea has both a horizontal and a vertical dimension. Many morphospecies are reported to have large depth distributions. In several of the discovered cryptic species complexes, the revealed species have been found to have different depth distributions on a scale of only hundreds of metres (Ekaratne *et al.* 1982; Nygren *et al.* 2005, 2010; Bleidorn *et al.* 2006; Luttikhuizen & Dekker 2010). Considering that the same species can be reported from the intertidal to several thousands metres of depth, it is relevant to ask how many species that could be involved. A study by Schüller (2011) on the morphospecies *Glycera kerguelensis* indicates that depth may have a major impact on species distribution. However, it remains to assess how the occurrence of cryptic polychaete species correlates with depth.

Apart from the fact that ignoring cryptic species leads to poor science, both basic and applied (Knowlton 1993), it moreover means that we underestimate the species richness in the sea. Discovering and describing this hidden part of biodiversity should be brought up on the current agenda as one of the major challenges in a world facing a biodiversity crisis (Bickford *et al.* 2007). Cryptic species are also important if we want to find areas of endemism, and thus have fundamental implications for conservation and management. One such example of an endangered cryptic species is *Hediste japonica* which distribution has been found to diminish at a fast rate (Sato & Nakashima 2003). Knowledge of the existence of cryptic species and their distribution is necessary for correct actions to be taken. Even if we still do not know the magnitude of cryptic diversity it is clear that morphology alone is far from enough for assessing biological diversity.

Molecular approaches

Even if it was possible to discover cryptic species through life history comparisons (Dehorne 1933; George 1967; Wilson 1983; Christie 1985), thorough morphological investigations (Westheide & Rieger 1987), and reproductive experiments earlier (Hauenschild 1951; Åkesson 1978, 1984; Weinberg *et al.* 1990, 1992; Rice 1991; Marsden 1992), it is only since molecular methods have become routinely used that we have begun to appreciate how common cryptic species are (Bickford *et al.* 2007). With some exceptions (Grassle & Grassle 1976; Paxton 1979; Robotti 1979; Rice & Simon 1980; Britton-Davidian & Amoureux 1982; Ekaratne *et al.* 1982; Guérin & Kerambrun 1984; Autem *et al.* 1985; Grassle *et al.* 1987; Mustaquim 1988; see Table S3), few polychaetes were analysed with molecular data before 1990s. By then it became common to use protein electrophoresis (allozymes and general protein patterns) for assessing species boundaries (Cadman & Nelson-Smith 1990; Baoling *et al.* 1991; Robotti *et al.* 1991; Manchenko & Radashevsky 1993, 1994, 1998, 2002; Fong & Garthwaite 1994; Schmidt & Westheide 1994; Bastrop *et al.* 1995; Abbiati & Maltagliati 1996; Bonse *et al.* 1996; Rodríguez-Trelles *et al.* 1996; Röhner *et al.* 1996, 1997; Sato & Masuda 1997; Gamenick *et al.* 1998; Jürss *et al.* 1999; Maltagliati *et al.* 2000, 2001, 2004; Scaps *et al.* 2000; Rouabah & Scaps 2003; see Table S3). In the late 1990s, fragment-based analyses like RAPD were added to the available methods, for polychaetes foremost used by the group around Westheide (Schmidt & Westheide 1998, 1999, 2000; von Soosten *et al.* 1998; Westheide & Hass-Cordes 2001; Westheide & Schmidt 2003; Kruse *et al.* 2003; Lewis & Karageorgopoulos 2008; see Table S3). In the last ten years, the majority of the published studies on cryptic polychaete species are based on discrete mitochondrial and/or nuclear sequence data (Schulze *et al.* 2000; Dahlgren *et al.* 2001; Chen *et al.* 2002; Hurtado *et al.* 2002; Breton *et al.* 2003; Glover *et al.* 2005; Jolly *et al.* 2005, 2006; Nygren *et al.* 2005, 2009, 2010; Bleidorn *et al.* 2006; Schulze 2006; Iannotta *et al.* 2007, 2009; Audzijonyte *et al.* 2008; Rice *et al.* 2008; Blake 2009; Blank & Bastrop 2009; Halt *et al.* 2009; Meißner & Blank 2009; Mahon *et al.* 2009; Pleijel *et al.* 2009; Virgilio *et al.* 2009; Wiklund *et al.* 2009a,b; Barroso *et al.* 2010; Capa *et al.* 2010, 2013; Luttikhuizen & Dekker 2010; Carr *et al.* 2011; Luttikhuizen *et al.* 2011; Nygren & Pleijel 2011; Schüller 2011; Borda *et al.* 2013; see Table S2). The obvious reasons are that sequence data now is much easier and inexpensive to produce and that there are several advantages in comparison to allozyme and RAPD data (Black 1993; Thorpe & Solé-Cava 1994; Jones *et al.* 1998). However, allozyme and fragment-based analyses make up a substantial part of the known cryptic polychaete species (see Table S1), and both methods have been successful in inferring cryptic polychaetes in a number of cases. Allozyme data have also been used in conjunction with mitochondrial sequence data in more recent studies to get the nuclear part of evolution (Audzijonyte *et al.* 2008; Barroso *et al.* 2010).

Several authors argue that combined approaches using as much data as possible is the best way when inferring species boundaries, including data on ecology, physiology, reproductive biology, reproductive incompatibility, morphology and molecular data (Lee 2004; Dayrat 2005; Schlick-Steiner *et al.* 2007; Lewis & Karageorgopoulos 2008; Rice *et al.* 2008; Halt *et al.* 2009; Capa *et al.* 2010; Nygren *et al.* 2010). However, much of these data are hard to come by and in most cases we are left with morphology and molecular data. In the case of cryptic species, morphology is usually not informative and we will have to rely on molecular data. However, when we use only gene sequences to infer species boundaries, it is necessary to use both mitochondrial and nuclear data to draw firm conclusions. A mitochondrial gene phylogeny needs not to be the species phylogeny due to, for example, lineage sorting or mitochondrial introgression (Nichols 2001; Ballard & Whitlock 2004). As we rarely know beforehand the level of intraspecific variation or the phylogeographical history of a species, divergent mitochondrial lineages might also be the result of retention of old haplotypes in subdivided populations within the same species (Audzijonyte *et al.* 2008). Thus, we need both data types to sort out what are intra- and interspecific differences. A single-gene approach as in the Barcoding of life-initiative may be an efficient way of detecting cryptic diversity (Carr *et al.* 2011), but will not be sufficient for species delineations (Ferguson 2002). We still do not have enough data to know how effective barcode identification of polychaetes with mitochondrial COI is. However, if we consider the examples on cryptic polychaetes in Table S1, where we have both mitochondrial and nuclear data to back up the number of inferred species, a barcode approach using the suggested 10× difference as a rule of thumb (Hebert *et al.* 2004; Carr *et al.* 2011), would identify the correct number of cryptic species in several (e.g. Pleijel *et al.* 2009; Nygren & Pleijel 2011; Schüller 2011) but not all of the examples

(Audzijonyte *et al.* 2008; Nygren *et al.* 2010). In addition to COI, there are also a few studies using alternative mitochondrial genes like Cytb, 16SrDNA and Cox3-trnQ-nad6 either in combination or as replacements (Bastrop *et al.* 1998; Dahlgren *et al.* 2001; Breton *et al.* 2003; Bleidorn *et al.* 2006; Jolly *et al.* 2006; Schulze 2006; Audzijonyte *et al.* 2008; Blank & Bastrop 2009; Halt *et al.* 2009; Iannotta *et al.* 2009; Nygren *et al.* 2009; Virgilio *et al.* 2009; Wiklund *et al.* 2009a,b; Capa *et al.* 2010; Luttikhuizen *et al.* 2011; Capa *et al.* 2013).

The ITS-region is the most commonly applied nuclear marker and it has been shown to be a useful marker for separation of cryptic species (Westheide & Hass-Cordes 2001; Chen *et al.* 2002; Iannotta *et al.* 2007; Halt *et al.* 2009; Nygren *et al.* 2009, 2010; Pleijel *et al.* 2009; Nygren & Pleijel 2011; Borda *et al.* 2013; Capa *et al.* 2013). As far as known differences between cryptic species is often substantial with both base pair-differences and indel events. However, cloning might be necessary when using the ITS-region, as there may be several hundreds of copies of ITS in one or several parts of the genome. Concerted evolution (Liao 1999) act to homogenise the copies but there may still be too much intra-individual variation for standard single PCR. In a few studies, the more conservative 18SrDNA and 28SrDNA (Meißner & Blank 2009; Nygren *et al.* 2009; Wiklund *et al.* 2009a; Schüller 2011) as well as the low-copy protein-coding gene Histone H3 (Nygren *et al.* 2009; Wiklund *et al.* 2009b), have been found to be useful for species separation.

Species concepts and their application in sympatry and allopatry

Even though there is a plethora of different species concepts (Mayden 1997) most of them agree that species are evolving metapopulations separated from other such metapopulations (Queiroz 2007). Queiroz argue that in the process where these metapopulations are separating they will acquire, for example, reciprocal monophyly, phenetic differences and reproductive incompatibility, although not necessarily in the same order or on the same time scale. The different species concepts, of which the morphological (MSC), phylogenetic sensu Mishler & Theriot (2000) (PSC), and biological species concept sensu Mayr (1969) (BSC) are three that are often referred to, differ in which of the aforementioned criteria need to be fulfilled. A morphological species concept is obviously not useful for cryptic species, but both the BSC and PSC are relevant. In practice, the BSC is rarely used explicitly in either separation of cryptic or non-cryptic species, as it is hardly ever tested whether members of two inferred species are able to reproduce. Instead, we deduce from morphological or genetic data that what we describe as separate species also are reproductively isolated groups. In sympatry, the PSC and BSC are equivalent. Distinct genetic lineages, reciprocally monophyletic for nuclear and mitochondrial markers, indicate the presence of reproductive barriers between the lineages (metapopulations).

Whereas the delineation of cryptic species in sympatry is straightforward, it is more difficult in allopatry since some differences are to be expected due to isolation by distance. Thus, geographically separated populations may be connected through a series of intermediate populations that were not sampled. Needless to say, it is thus of great importance that as much as possible of the distribution area is sampled when assessing species boundaries. There is also the possibility that two separately reciprocally monophyletic metapopulations without any current gene flow (that would be regarded as separate species according to the PSC) still are potentially interbreeding (and would be regarded as conspecifics according to the BSC). It has been argued that the time necessary for allopatric populations to acquire reciprocal monophyly as observed from multiple types of data (mitochondrial, nuclear) is more than enough for acquiring reproductive isolation (Avise & Wollenberg 1997; Knowlton 2000), but this assumption has to my knowledge not been tested. For allopatric cryptic species, the question will thus always be how much difference is enough, and to that question there may be no easy answer. But this is not unique to molecular data, but applies to morphology as well, that is, how much difference in, for example, details of chaetal morphology is enough for species recognition (Westheide & Schmidt 2003)?

Based on what we know from studies that combine genetic data with reproductive crosses, there will probably never be a universal yardstick (Ferguson 2002) of how much genetic differentiation is enough for species recognition. In *Streblospio*, a 5% difference in COI between populations from Southern Florida and the Western Gulf of Mexico was not enough for any reproductive incompatibilities while 15% difference between the two major, partly sympatric, clades meant complete reproductive isolation (Schulze *et al.* 2000). In the sympatric cryptic species-pair *Ophryotrocha japonica* and *O. glandulata*, a 5% difference was enough for full reproductive isolation (Wiklund *et al.* 2009b; Paxton & Åkesson 2010). The relationship between degree of reproductive compatibility and phylogenetic relationship is not a simple one. It is not necessarily the closest species in a cryptic species group that demonstrate the highest degree of reproductive compatibility (Rice *et al.* 2008; Wiklund *et al.* 2009b; Paxton & Åkesson 2010). An old and relevant question is also if the ability to interbreed should be the final and decisive criteria for species recognition, the ability to interbreed is after all the plesiomorphic condition (Rosen 1979).

However, if possible to conduct, a reciprocal reproductive compatibility test is an informative piece of evidence for species demarcation, and, in combination with a molecular analyses, it can give important insights into the evolution of reproductive barriers.

How old are cryptic species complexes?

The genetic differences between cryptic polychaete species are in many cases substantial. This was already noticed for allozyme data where cryptic species often had large genetic differences with fixed diagnostic loci (allozyme data) or diagnostic bands (RAPD data) (e.g. Rodríguez-Trelles *et al.* 1996; Manchenko & Radashevsky 1998; Jürss *et al.* 1999; Westheide & Schmidt 2003; see Table S3 for more examples). The same is true for sequence data where the COI-distances (p-distance, K2P-corrected) often are larger than 15% (e.g. Iannotta *et al.* 2007; Pleijel *et al.* 2009; Schüller 2011; see Table S2 for more examples). If we use the molecular clock by Chevaldonné *et al.* (2002), calibrated on vent polychaetes that were separated by the disruption of the Farallon-Pacific Ridge with divergence rates estimated to 0.26–0.50% per million years, many cryptic polychaete species complexes would be older than 30 million years (Nygren *et al.* 2009; Wiklund *et al.* 2009a; Luttikhuizen & Dekker 2010). However, a molecular clock based on polychaetes living in extreme environments might not be representative for other polychaetes. An alternative that is also applied in several studies of cryptic polychaetes (Westheide & Schmidt 2003; Jolly *et al.* 2006; Iannotta *et al.* 2007, 2009; Barroso *et al.* 2010) is to use the divergence rate from transisthmian geminate pairs of snapper shrimps that were separated at the time of the closure of the Panama Isthmus. Knowlton *et al.* (1993) first estimated the divergence rate to be 2.2–2.6% per million years, but later revised their estimate to 1.4% (Knowlton & Weigt 1998), while Hickerson *et al.* (2003) provided yet another calibration on the same data set taken coalescent theory into account and estimated the divergence rate to be somewhere between 1.98 and 2.91% per million years.

Apart from geological events like the disruption of the Farallon-Pacific ridge or the closure of the Panama Isthmus, fossils are an alternative way for calibration of a molecular clock. Even if there are few fossils of polychaetes, there are fossils of *Ophryotrocha* jaws that are similar to recent jaws in the cryptic *O. labronica* complex, and these are dated to 85 million years old (Eriksson & Lindström 2000). If we assume that these jaws represent the most recent common ancestor to the living members of the *O. labronica* group, the divergence rate for COI would be 1.9% per million years. Regardless if the fastest or slowest of these rates are applied, most of the cryptic polychaetes for which COI-data are available were already present long before the onset of the last period of recurrent ice ages that started 1.8 million years ago. If the molecular clock for polychaetes is within the specified limits, it would mean that among the known cryptic polychaete species there are no obvious examples of relatively recent speciation.

Are some polychaetes more cryptic than others?

It would be reasonable to assume that cryptic polychaetes are more likely to be found in certain types of polychaetes, for example, among those with a relative simple morphology (Barroso *et al.* 2010), or among those that live in extreme environments where harsh abiotic factors would favour stabilising selection and lead to morphological stasis (Bickford *et al.* 2007; Borda *et al.* 2013), or among polychaetes that are reported to be widely distributed despite having direct development or short-lived larvae (Schmidt & Westheide 2000; Westheide & Hass-Cordes 2001). It has also been suggested that colour polymorphism or poecilogony (more than one mode of development) in one and the same nominal species would be reason to suspect that there is more than one species involved (Hoagland & Robertsson 1988; Nygren & Pleijel 2011). Is it possible to discern any common pattern from the known cryptic species?

One issue that has to be considered before such an evaluation is that the examples in Table S1 are not random and that the list merely reflects what kind of polychaetes that have been investigated. There is no coincidence that several of the known cryptic polychaete complexes are bioindicator species (referenced above), because these are among the best-studied polychaetes. For many of the polychaetes, it is also true that other types of data, such as colour variation, have lead to a genetic investigation, and thus the discovery of cryptic species (Nygren *et al.* 2005; Nygren & Pleijel 2011). The examples in Table S1 also represent the cases where cryptic polychaetes *have* been found through genetic studies. For a more fair comparison, it is necessary to consider those studies where several species have *not* been found in, for example, widely distributed taxa, or among colour polymorphic species (e.g. Meyer *et al.* 2008; Nygren *et al.* 2011; Ahrens *et al.* 2013).

With these considerations, what conclusion can be drawn from the data available on cryptic polychaetes? First, neither simple-bodied polychaetes nor polychaetes living in extreme environments is better represented than morphologically more complex types of polychaetes, or polychaetes living in more normal habitats. Cryptic species do neither seem to be more common among polychaetes with direct development or short-lived larvae than among those with planktotrophic larvae, regardless if they are widely distributed or occurring sympatrically. Several species have also been shown to have a wide geographical distribution despite short-lived larvae or direct development (Westheide *et al.* 2003; Schulze 2006; Meyer *et al.* 2008). In short, species need not to have large distribution areas with certain types of larval development to be cryptic species candidates, and species need not to be cryptic complexes even if they have a large distribution (Ahrens *et al.* 2013). Further, colour polymorphism and poecilogony are often found to indicate cryptic species (Paxton 1979; Bonse *et al.* 1996; Manchenko & Radashevsky 2002; Kruse *et al.* 2003; Nygren *et al.* 2005, 2010; Iannotta *et al.* 2009), but not always in a simple way (Schulze *et al.* 2000; Pleijel *et al.* 2009; Nygren & Pleijel 2011) and neither colour polymorphism nor poecilogony is obligately indicative of cryptic species (Gibson *et al.* 1999; Morgan *et al.* 1999; Rice & Rice 2009; Nygren *et al.* 2011; Kesäniemi *et al.* 2013). In conclusion, there are few, if any, generalisations that can be made about which type or types of polychaetes that are most likely to harbour cryptic diversity.

Should cryptic species be described?

Even though cryptic species routinely are found in genetic surveys few of them are formally described (Westheide & Schmidt 2003). This is unfortunate because unnamed species are rarely taken into consideration in biological investigations or management programmes. The reluctance to naming species based on molecular information may have several explanations (Westheide & Schmidt 2003). One of the major issues is to sort out the taxonomy. Many cryptic species complexes are veritable nightmares for a taxonomist (Knowlton 1993) with many available names that can be applied for the discovered genetic lineages (Schlick-Steiner *et al.* 2007). Thus, the sampling strategy should incorporate type localities for as many of the available names as possible.

Newly collected topotypes, type material when present, as well as original descriptions may help to allocate the names to the correct clade (Sato & Nakashima 2003; Sikorski & Bick 2004; Nygren *et al.* 2005, 2010; Lewis & Karageorgopoulos 2008; Nygren & Pleijel 2011). However, in many cases, it will probably not be possible to refer all if any names to a certain clade. This leaves two choices, either to use the oldest available names in a pragmatic way, or to treat the old names as *nomina dubia*. Lost type material, how crude it may sound, may be a golden opportunity, because it allow the taxonomist to designate a neotype that can be sequenced and provide an unequivocal reference point to the species name.

Another critical issue is how the genetic information can be incorporated into the species diagnosis and description. Several authors have used an apomorphic approach where diagnostic fragments (RAPD), or inferred diagnostic genetic changes in the phylogeny, serve the role of diagnosing the species (e.g. Westheide & Hass-Cordes 2001; Halt *et al.* 2009) while Nygren & Pleijel (2011) used a node-based approach where the actual sequences in combination with the tree topology and distance measures served to diagnose the species. The actual name-bearer of the species name is the holotype, but instead of using only morphological characters in the diagnosis as in conventional descriptions, the information from the genetic data is also included and used for species separation.

Conclusions

Already Grassle (1980) asked the question 'How common are cryptic polychaetes' when she and her husband (Grassle & Grassle 1976) had discovered six cryptic species of *Capitella capitata* after an oil spill in West Falmouth, Massachusetts in September 1969. Even if we today have many more examples of cryptic polychaetes we do not yet have the answer to that question. We are just in the very beginning of understanding the magnitude of this phenomenon, as the examples we have on cryptic polychaetes only represent a small fraction of all described morphospecies of polychaetes. Thorough worldwide genetic studies of cosmopolitan distributed polychaetes are few as are comprehensive genetic studies of species distributions in general.

In a study by Nygren & Pleijel (2011), five different species of *Eumida sanguinea* were found in boreal waters, and when the Mediterranean was included another five different species could be identified. Considering that *E. sanguinea* is reported to be a cosmopolitan species, how many additional species of '*E. sanguinea*' are present worldwide? Will we discover similar patterns in many other polychaetes? Other examples (Blake 2009; Bleidorn *et al.* 2006; Borda *et al.* 2013; Jolly *et al.* 2006; Lewis & Karageorgopoulos 2008; Meißner & Blank 2009) indicate that this might be a relatively common phenomenon. If our knowledge is fragmentary at best in the temperate shallow waters, the situation in the deep sea as well as in most other parts of the world including the highly diverse tropical regions is even worse. In addition to that we have a poor understanding of the horizontal distribution of polychaetes we are in many cases not aware of how large their actual depth distribution are. Both examples from the shallow waters and the deep sea indicate that depth may play an important part of species distribution but much work remains before a clear picture can emerge.

Cryptic polychaetes are not restricted to widely distributed nominal species, but are often found in sympatry, often in the same habitat or in very close vicinity. In Pleijel *et al.* (2009), three cryptic species of *Gyptis* was found in the same homogenous habitat in an area <100 m^2, in Nygren & Pleijel (2011) five cryptic species of *Eumida sanguinea* was found in the same piece of coralligene, and in Christie (1985) three cryptic species of *Chaetozone setosa* were identified in three sites spanning 65 kilometres. How can so many morphologically similar species coexist in the same habitat? In *Capitella* (Grassle & Grassle 1976), it could be shown that the species presence were partly separated in time, and in others ecological differences might be invoked for the sympatric existence (Blank *et al.* 2006; Paxton 1979; Kikuchi & Yasuda 2006; Nygren *et al.* 2010). A related question to the sympatric occurrence of many cryptic species is where and how they did originate. Are most of them the result of vicariant processes and dispersal (Glasby 2005), or is it necessary to invoke other explanations (Maltagliati *et al.* 2004)?

With limited resources, how do we best spend our money and time if we want to unravel and describe cryptic biodiversity in polychaetes? There are two possible routes that can be followed, but which should be seen as complementary. One path leads towards large-scale analyses where whole samples are sequenced. By dividing, for example, grab samples into two equal parts, one half can be sorted and determined the traditional way by skilled taxonomists, the other half can be sequenced with next-generation sequencing (Fonseca *et al.* 2010). By targeting a number of specific genetic markers like COI and more conservative regions like 28S, the number of distinct genetic lineages can be matched with the number of morphologically identified polychaete lineages. An alternative way would be to morphologically identify and sequence each specimen in the samples individually. In this manner, we will get a rough idea of the magnitude of cryptic biodiversity of polychaetes, and in which taxonomic groups they are most likely to be found.

The second path leads to investigation of possible cryptic species-groups sampled from as much of their distribution area as possible, including the type localities. The morphology of each specimen sampled is studied and documented in detail, to make it possible to check all specimens also after the genetic analyses. Ecological and behaviour data are thoroughly noted. At least one mitochondrial and one nuclear marker should be sequenced, and if several different genetic lineages that may be referred to as different species are found, the taxonomy of the species-group should be sorted out and the different cryptic species assigned proper names. In this way, we will with time have numerous case-studies where we will have learned a lot on species-distributions, intra- and interspecific differences, habitat and depth preferences, variation in colouration and other subtle details that are lost during standard collecting procedures.

Knowlton (2000) strongly argued that we should spend more time out in the field observing our study objects, and stated ‘two weeks in the field may be worth two years in the laboratory’. This is equally applicable today. The powerful molecular tools we now have in our hands will in combination with morphological and ecological studies provide a firm foundation for the unravelling, description and explanation of this hidden and unexplored dimension of life on earth.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Polychaetes where molecular data has been used to identify cryptic diversity.

Table S2. Polychaetes where discrete sequence data has been used to identify cryptic diversity.

Table S3. Polychaetes where other types of molecular data, except discrete sequence data, have been used to identify cryptic diversity.

References S1. References for supporting information.